CORRELATION OF BIOLOGICAL CHARACTERISTICS OF SMOLTS WITH SURVIVAL AND TRAVEL TIME

Annual Report FY 1987

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Project No. 87-406
Contract No. DE-AI79-87BP35245

,June 1988

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ABSTRACT

The biological characteristics of smolts were examined to determine their effect on estimates of survival in the Columbia and Snake rivers. Freeze branded groups of steelhead trout (Salmo gairdneri) from Lyons Ferry state Fish Hatchery (SFH) and Wells SFH and spring chinook salmon (Oncorhynchus tshawytscha) from Winthrop National Fish Hatchery (NFH) were used to estimate survival. Past estimates of survival, using a ratio of test and control fish recaptured at McNary Dam, have resulted in estimates > 100%, presumably due to some unknown bias. Study objectives were to determine if stress and descaling, degree of smoltification, and prevalence of bacterial kidney disease (BKD) differed among test and control groups of fish, thereby biasing survival estimates.

Plasma cortisol, plasma glucose, and a handling-stress challenge were used to assess the stress response of fish in test and control groups. Fish trucked long distances (1.5 to 2.5 h) to release sites were no more stressed than those trucked short distances (about 0.5 h), but both groups were more stressed than those released directly from hatchery raceways. Normal marking and release operations were not particularly stressful to the fish, but deviations from protocol did result in stressful conditions. Dissimilar plasma glucose responses in steelhead occurred in two groups that had erroneous survival estimates for steelhead of about 130%.

Gill Na^+K^+-ATPase, plasma thyroxine, fish morphology, and plasma ions after 24-h Seawater challenges were used to compare the extent of smoltification in control and test fish. Groups were similar at the hatcheries, but were different after the migration to McNary Dam. Gill ATPase activity increased 2-3 fold during the first 20 days of migration. Smoltification of steelhead from Lyons Ferry SFH was unique; exhibiting the least smolt development at the hatchery and greatest difference between test and control groups at McNary Dam. Survival estimates greater than 100% for steelhead originating at Lyons Ferry were attributed to the differences in smolt development of test and Control groups at McNary Dam.

Prevalence of BKD in spring chinook from Winthrop NFH decreased between time of release and time of recapture at McNary Dam. Later migrants, when compared to others in a release group, were more prone to test positive for BKD using enzyme linked immunosorbant

assay (ELISA) suggesting smolts with acute BKD infections had relatively low migration rates.

Recommendations are to implement a program of quality control to ensure that stress, smoltification, and disease of all groups is similar and, if not, to determine where the differences exist. AlSO recommended is discontinuing use of control groups transported directly from the hatchery because these smolts are markedly less developed when compared to test groups with longer inriver migration time.

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INTRODUCTION

Information on the migrational characteristics and survival of juvenile salmon (*Oncorhynchus* SP.) and steelhead trout (*Salmon gairdneri*) in the Columbia River Basin is collected by the Fish Passage Center through the smolt Monitoring Program. This information is used primarily to assist in management and evaluation of the Water Budget, a volume of water set aside for the enhancement of environmental conditions for seaward migrating smolts. Implicit in the water Budget concept is that travel time of smolts can be decreased and their survival can be increased by increasing flows.

Indices of travel time and survival of smolts are determined in selected reaches of the Columbia and Snake rivers for use in management and evaluation of the Water Budget. The Water Budget Managers have recognized that in addition to river flows, fish health and physiological status may account for unexplained variability in the travel time and survival estimates. For example, bacterial kidney disease (BKD) is common among spring chinook salmon (0. *tshawytscha*) at some hatcheries, but it is not known to what extent this disease may account for post-release mortality observed during seaward migration. Prior to 1987, no information was collected on the prevalence of disease or physiological characteristics of the groups of smolts used to estimate travel time and survival.

During 1985 and 1986 some steelhead survival estimates were obviously in error with values of over 100% estimated through the index reach. The water Budget Managers acknowledged that such bias may have resulted from violations in the assumptions necessary in making the computations. The survival estimates were calculated as the ratio of the proportion of marked test and control fish recovered at a downstream collector dam. Test groups were released at the upstream end of the reach and control groups were released at the downstream end, above the recovery site. We proposed that the following two important assumptions may have been violated using this strategy:

Assumption 1: Test and control fish are identical at time of release.

Assumption 2: Test and control fish are identical at the recovery site, McNary Dam, and therefore equally susceptible to collection at the site.

In fact, several mechanisms may be responsible for differences in the test and control fish which could result in biased estimates.

The experimental design requires that control groups be transported by truck for as long as 3.5 h while test fish are either not transported or transported only a short distance (Figure 1). Therefore, the first assumption may be violated if control groups experience transportation-related stress because they are trucked to remote release sites. Recent studies have established that handling and transporting fish causes stress and that repeated stressors have a cumulative effect on the fish (Specker and Schreck 1980; Wedemeyer et al. 1984; Barton et al. 1986).

The second assumption may be violated if fish passing the downstream recovery site, such as McNary Dam, are at different stages of development. Smolt development, i.e. smoltification, occurs prior to and during the seaward migration of juvenile salmonids (Folmar and Dickhoff 1980; Zaugg et al. 1985). Among fish of hatchery origin, smolts appear to be more developed after completing a longer time or distance of inriver migration. Accompanying the development are physiological, morphological, and behavioral changes that may increase the migration rates and collection rates of test groups. As a result, downstream from the release site of the control fish (Figure 1) the test fish may have a higher migration rate resulting in better survival to McNary Dam. In addition, Giorqi et al. (1988) suggest there is a relationship between physiological status of smolting yearling chinook salmon and their susceptibility to guidance by traveling screens. In this case such a relationship may result in a greater proportion of the more smolted test fish being collected, and thereby biasing the survival estimate.

The attributes of smolts monitored durinq spring 1987 can be divided into three general categories: 1) stress and descaling, 2) stage of smoltification, and 3) prevalence of disease, i.e. bacterial kidney disease.

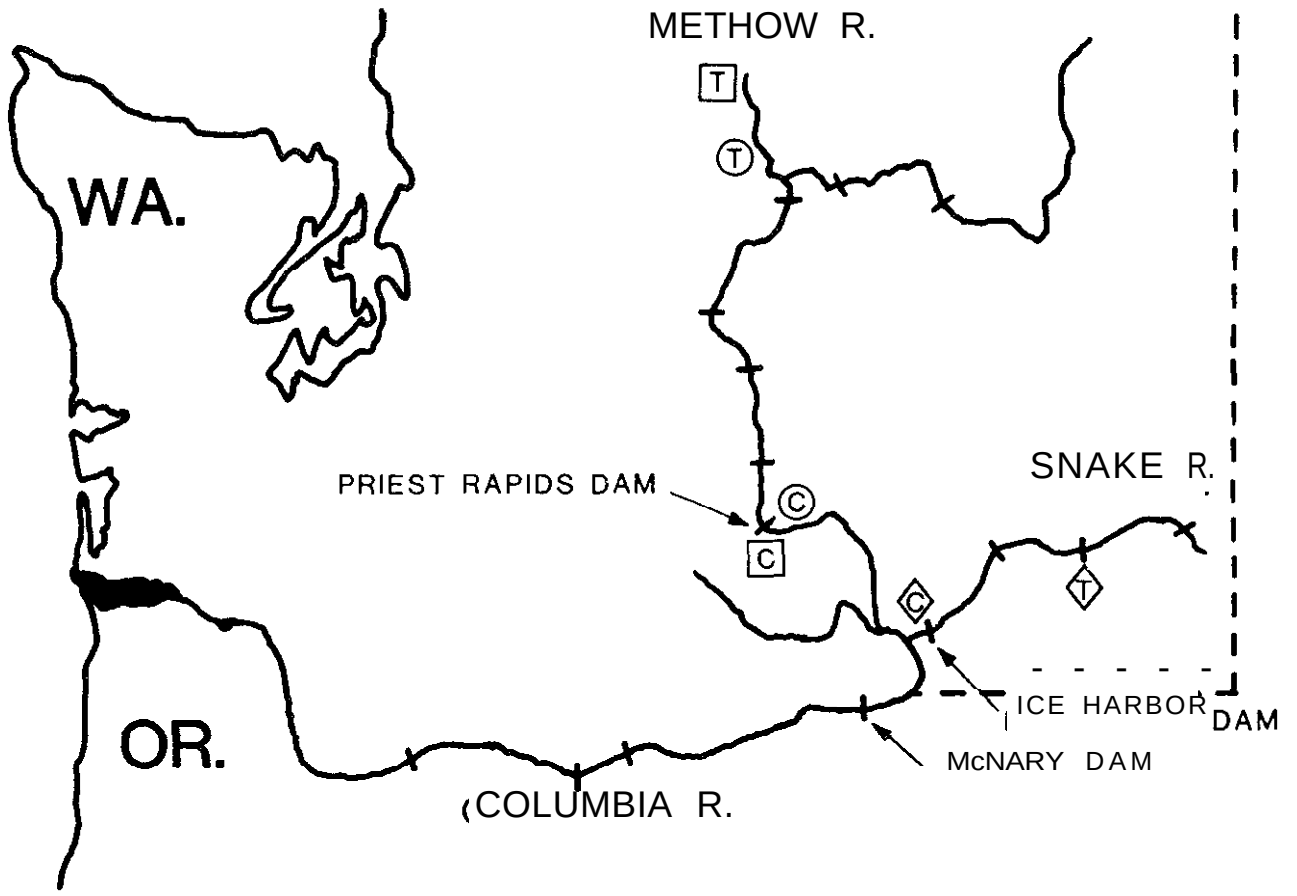

Figure 1. Map of the Columbia Basin with release locations of test and control groups of fish from Lyons Ferry SFH (Ⓣ, Ⓒ), Wells SFH (Ⓣ, Ⓒ), and Winthrop NFH (T, C), 1987.

GOAL AND OBJECTIVES

The goal of this project was to collect data on the biological characteristics of smolts to compliment the environmental data currently collected for analysis of travel time and survival. In the short-term, these data will be used by the Water Budget Managers to evaluate assumptions and methods used in making survival estimates. The following objectives and the respective hypotheses were tested to attain the short-term goal:

Objective 1: Determine if stress or descaling associated with the release of test and control groups of smolts is different.

HO: Transportation and release of control fish is no more stressful than modes of releasing test fish.

Objective 2: Determine if test and control fish are at different stages of smoltification at release or upon leaving the index reaches.

Ho: Fish in test and control groups used to estimate survival are not at different stages of smoltification.

Objective 3: Estimate the prevalence of BKD infection in groups of yearling spring chinook salmon smolts at release and at recapture at index reach collection facilities to determine if mortality of fish with chronic infection of BKD may account for high inriver mortality.

Ho: The proportion of spring chinook salmon with chronic infections and high antigen levels for BKD is the same at release and at recapture downstream.

METHODS

Study sites and fish collection

Fish used in this study were collected from marked groups used to estimate survival in the Mid-Columbia and the lower Snake rivers as part of the 1987 Smolt Monitoring Program. These fish included steelhead from Lyons Ferry SFH, Washington Department of Wildlife; steelhead from Wells SFH, Washington Department of Wildlife; and spring chinook salmon from Winthrop NFH, U.S. Fish and Wildlife Service.

The fish were marked using freeze branding techniques (Miqhell, 1969), as described in more detail by the Fish Passage center (1988). Steelhead were marked at Lyons Ferry SFH about 2-3 weeks prior to release, and at wells SFH about 1-2 days prior to release. Spring chinook salmon were marked at Winthrop NFH about 6 months prior to release.

Each survival estimate was based on a test and control group of fish. Three replicate releases of test and control groups were made at four day intervals resulting in three survival estimates for the stock of each hatchery (Table 1). All groups of fish were sampled prior to release, hereafter termed prerelease samples; at the time of release; and at the downstream recapture site, McNary Dam (Table 2). Prerelease samples were taken before fish were subjected to disturbances related to hatchery release procedures (e.g. raceway drawdown, crowding, etc.) and prior to daily activities such as feeding. At release, samples from the test groups were collected either after drawdown of the water level in the raceway at Winthrop NFH or from the truck for the other hatcheries. Release samples from all control groups were collected directly from the truck.

At the McNary Dam fish collection facility each group was sampled at about the 25, 50, and 75th percentile of their passage past the dam, referred to in this report as early, middle, and late samples from the migration. To determine collection dates for the 25, 50, and 75th percentile the cummulative percentile of passage was estimated on a daily basis for each of the 18 branded groups. Cummulative percentile of passage

Table 1. Hatchery of origin and release locations for test and control groups used to estimate survival and sampled to assess extent of stress, descaling, and smoltification in each replicate.

Release Dates	Group	Release Mode	Release Site
Steelhead - Lyons Ferry SFH			
Replicate 1 = 4/23/87	control	truck	below Ice Harbor Dam
	test	truck	hatchery
Replicate 2 = 4/27/87			
Replicate 3 = 5/01/87			
Steelhead Wells SFH			
Replicate 1 = 4/23/87	control	truck	below Priest Rapids Dam
	test	truck	Methow River
Replicate 2 = 4/27/87			
Replicate 3 = 5/01/87			
SP. chinook - Winthrop NFH			
Replicate 1 = 4/20/87	control	truck	below Priest Rapids Dam
	test	raceway	hatchery
Replicate 2 = 4/24/87			
Replicate 3 = 4/28/87			

Table 2. Study design showing biological characteristics monitored, indices used, and time of sampling of juvenile steelhead from Lyons Ferry SFH and Wells SFH and juvenile spring chinook salmon from Winthrop NFH.

	PRERELEASE (Hatchery)	RELEASE	RECOVERY (McNary Dam)
STRESS			
Basal			
Cortisol	x	x	
Glucose	x	x	
Handling-stress challenge			
Cortisol	x	X	
Glucose	x	x	
DESCALING	X	x	
SMOLTIFICATION			
Gill ATPase	x		x
Thyroxine	X		x
Morphology	x		x
Seawater Challenge (Steelhead only)			x
DISEASE			
BKD (Chinook only)	x		x

was estimated by determining the year-to-date-cummulative-number of smolts collected from each brand group and dividing by the total number of fish of each brand expected to be recaptured during the year based on previous years. Branded fish were removed from the collection system at McNary Dam after the brands were recorded for the smolt Monitoring Program.

Fish sacrificed for plasma or tissues were collected with a dip net and transferred into a 20 L bucket containing 150 mq • L^{-1} tricaine methanesulfonate (MS-222). After the fish were anesthetized, the caudal peduncle was severed and blood was collected in a heparinized capillary tube. Blood was then centrifuged, the plasma was removed and stored in liquid nitrogen at -196°C until brought back to the laboratory and stored at -78°C.

Stress and Descaling

Fish were sampled at prerelease and release to assess the degree of stress and descaling. Prerelease samples were used to establish the baseline conditions of stress indicators and descaling. Plasma cortisol and glucose were selected as primary and secondary stress indicators, respectively, as measures of the severity of the response to stressors.

For each test and control group, 20 fish were sampled at prerelease and release to determine baseline levels of plasma cortisol and glucose. I" addition to sampling fish in a "resting' state for the baseline, a handling-stress challenge was used to assess ability of fish to respond to a standardized stress. Twenty fish were sampled to determine the response to the handling-stress challenge test at prerelease and at release. In this challenge, fish were netted, held out of water for 30 seconds, and placed in water for one hour before they were anesthetized and blood was sampled. Subsequent stress responses to the challenge were determined by levels of plasma cortisol and glucose.

Plasma cortisol was analyzed using the radio-immunoassay approach of Redding et al. (1984). Plasma glucose was analyzed using the hexokinase enzymatic method available from Sigma Diagnostics, St. Louis, Mo. As a quality assurance, standards were analyzed at regular intervals during the assays. The data were analyzed using analysis of variance (ANOVA) and the Student-Newman-Keuls (SNK) multiple range test. Use of

the term significant in the text refers to a statistically significant difference at the probability $P \leq 0.05$.

The percentage of fish classified as descaled or partially descaled was determined on samples of 100 fish at prerelease sampling and again at release using the criteria of the Fish Transportation and Oversight Team (FTOT). Partially descaled fish were identified as having at least one side of the fish with Scales missing from > 3% of the total scaled area. Statistical comparisons were accomplished using Chi-Square tests, (Sokal and Rohlf 1981). Significant differences refer to the probability $P \leq 0.05$.

Stage of Smoltification

Selected indicators of smoltification were determined for 20 fish in each test and control group in the prerelease samples and for 20 fish at the 25, 50, and 75th percentile of passage at McNary Dam. Gill Na^+K^+-ATPase levels, plasma thyroxine (T_4), plasma ion levels in a 24-h seawater challenge test, and body morphology were used to evaluate the level of smoltification. Gill filament samples were taken and assayed for Na^+K^+-ATPase using the method of Zaugg (1982). Thyroxine samples were assayed using the radioimmunoassay described by Dickhoff et al. (1978) and performed by Biotech Biochemical Analyses, Corvallis, OR. The data on ATPase and thyroxine were analyzed using ANOVA and SNK and statistical differences reported here refer to the probability $P < 0.05$.

Morphological characteristics of test and control groups of fish were compared as they passed McNary Dam. Photographs were taken of each fish for subsequent measurement of selected characteristics (Figure 2) using methods similar to Winans (1984). A digitizer was used to measure line segments on the photographs and, using a scaling factor, the actual length of each line segment was reconstructed in a record for each fish. The data was analyzed using the multivariate analysis of variance (MANOVA) of the SAS Institute (1985). Wilks' Lambda was used as the test statistic and statistical differences between morphology of test and control fish refers to probability $P < 0.05$.

seawater challenges were used to assess the osmoregulatory ability of test and control groups of steelhead as they passed McNary Dam. seawater challenges test the ability of smolts to regulate Na^+

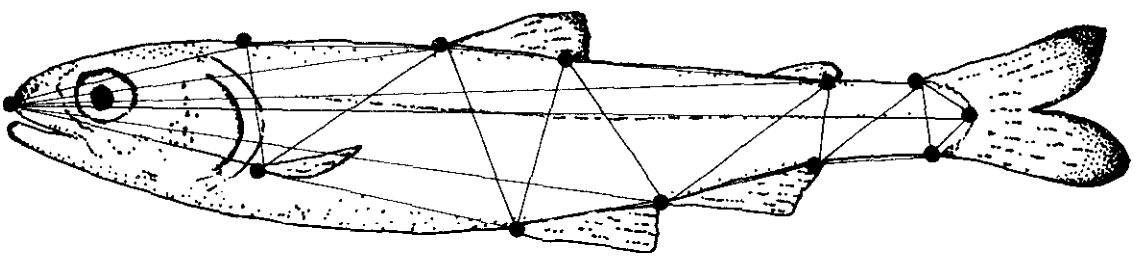

Figure 2. Schematic of smolt with points identifying line segments measured for morphological analysis.

and other ions that are encountered upon seawater entry. The ahility to maintain relatively low ion concentrations 1n the blood plasma despite the presence 0f high ion concentrations in the seawater environment is one characteristic of a fully functional smolt. We reasoned that any differences in the degree of snoltification of test and control fish at McNary Dam would be reflected in their ahility to osmoregulate. Only steelhead were subjected to seawater challenges because previous erroneous survival estimates for steelhead indicated that differences between test and control qroups were most likely to occur in these fish.

Steelhead for the seawater challenge tests were collected from the McNary Dam fish collection facility and held for a 24-h acclimation period. Fish were placed in a 500 L artificial stream tank with both a 20 $L \cdot min^{-1}$ flow from the Columbia River and a recirculating pump and charcoal water filter. one group of 20 steelhead was collected from each control group, and because they took longer to migrate past McNary Dam, two groups of 20 steelhead were collected from each test qroup. After a 24-h acclimation period the fish were rapidly transferred to a similar tank containing 30 parts per thousand (ppt) salinity of Instant Ocean[1], from Aquarium Systems, Mentor, Ohio. The water was recirculated and chilled to match the river water temperatures in the acclimation tank.

After 24 hours in seawater the steelhead were removed and placed in 150 pp" MS-222. Gill filaments for ATPase and blood plasma for osmolarity, Na^+, Cl-, and K+ ions were collected as described earlier. Osmolarity was determined using a vapor pressure osmometer. Sodium and K+ concentrations in the plasma were determined using a flame photometer. Chloride concentrations were determined using a chloridometer. Samples were analyzed in a random order with known standards at regular intervals during the assays. Data was analyzed using ANOVA, SNK, and Pearson correlation analysis and statistically significant differences were reported when $P \leq 0.05$.

[1] Reference to trade names does not imply endorsement by the Federal Government.

BKD in chinook salmon

Samples for RKD were take" from spring chinook at prerelease and recovery. Kidney and spleen tissue samples were collected and stored in microcentrifuge tubes in liquid nitrogen. Tissue samples were mashed and smears analyzed using the florescent antibody technique (FAT) with a 100 field count (McDaniel 1979). Samples were divided into one of four categories depending on the number of BKD conjugates as follows: neqative, none found; low 1-15; medium 16-90; and high, greater than 90. The same material was analyzed for antigen levels using the enzyme linked immunosorbent assay (ELISA). ELISA results were considered positive for BKD when optical densities were above 0.090.

RESULTS

Steelhead from Lyons Ferry SFH

Stress and descaling: At release, plasma cortisol levels of test groups of steelhead from Lyons Ferry SFH were significantly higher than levels in control groups (Figure 3). In this case release of test and control groups differed in two ways. First, test groups were hauled for about 15 min compared to 1.5 h for control groups; and second, test fish were transported at 1.0 $lb \cdot gal^{-1}$ compared to 0.8 $lb \cdot gal^{-1}$ for the control groups

Levels of cortisol in steelhead at release were significantly higher than levels in prerelease samples collected from undisturbed fish in raceways. Mean cortisol levels in prerelease samples ranged from 6.3 to 31.0 $ng \cdot mL^{-1}$ and from 68.5 to 119.8 $ng \cdot mL^{-1}$ in samples at release. Cortisol levels increased to a greater extent in the test fish at release than in controls in replicates one and three, whereas the increases were similar in the second replicate (Figure 3; Appendix 1).

Fish in test and control groups of release two were unusual in that they exhibited no increase in plasma qlucose at the time of release compared to prerelease samples (Figure 3; Appendix 2). An increased level of plasma glucose, indicative of a secondary stress response, is considered a normal response to stressful conditions, such as being crowded and loaded on the truck.

The handling-stress challenge was expected to result in an increase in plasma cortisol levels, such as those observed in prerelease challenges of replicates one and three (Figure 4). Basal levels of plasma cortisol in the prerelease samples from test and control groups ranged from 6.3 to 31.0 $ng \cdot mL^{-1}$. After the handling-stress challenge they ranged from 77.2 to 144.6 $ng \cdot mL^{-1}$. The cortisol response of steelhead in the control group of release two was unusual in that cortisol levels did not increase as much as levels in test group two (Figure 4; Appendix 1).

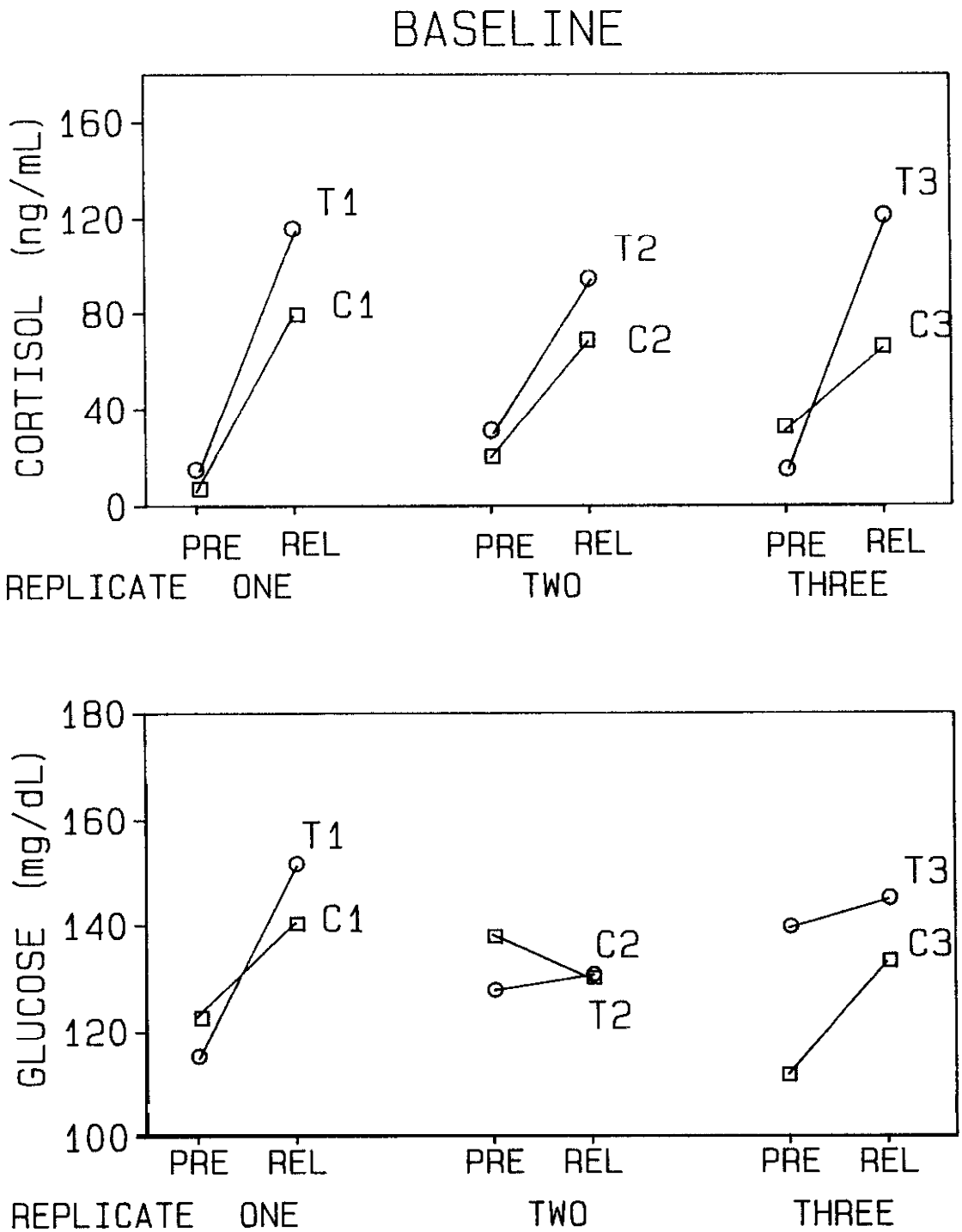

Figure 3. Mean baseline levels of plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) of juvenile steelhead in prerelease (PRE) samples collected at Lyons Ferry SFH and in samples collected at release (REL) sites.

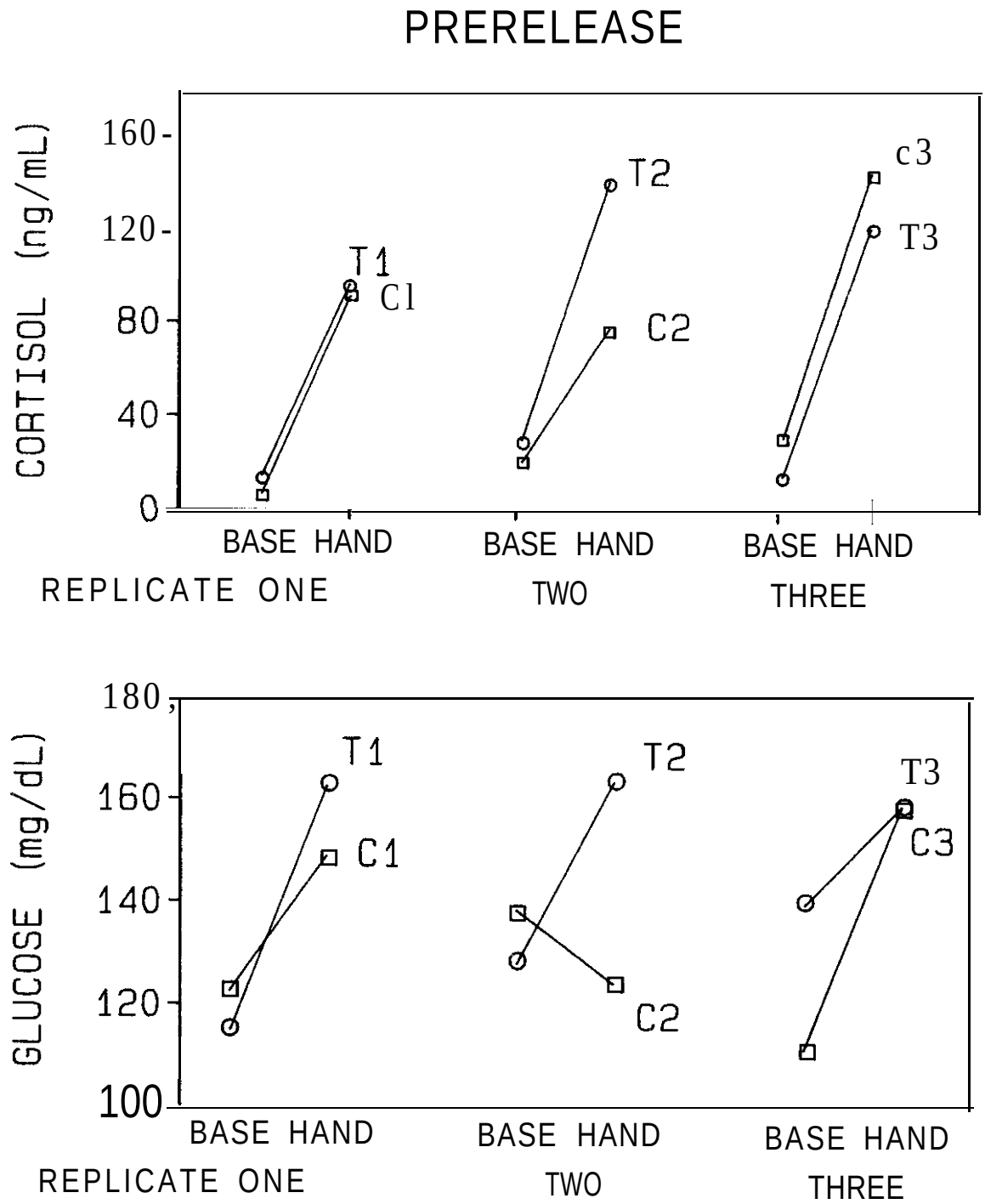

Figure 4. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile steelhead from Lyons Ferry SFH before (BASE) and one hour after a prerelease handling-stress challenge (HAND) performed prior to loading at the hatchery.

Steelhead were also expected to respond to the prerelease handling-stress challenge with increased levels of plasma glucose. Among prerelease samples all test and control groups had significantly higher plasma glucose after the handling-stress challenge except the control group Of release two in which plasma glucose declined in response to the challenge (Figure 4; Appendix 2).

The change in plasma cortisol levels in response to the handling-stress challenge at release was consistently greater among the control groups compared to the test fish of the same replicate (Figure 5; Appendix 1). The greater response in the control groups was the result of lower basal levels and slightly higher levels after the handling-stress challenge.

At release, the handling-stress challenge did not result in increased levels of plasma glucose, but rather a significant decline in test and controls of replicates one and three (Figure 5; Appendix 2). Plasma glucose of steelhead in test and control groups of replicate two did not change significantly in response to the handling-stress challenge.

stress responses of steelhead as indicated by plasma cortisol and glucose were most similar in replicates one and three. Survival estimates were 103, 131, and 108% for replicates one, two, and three, respectively (Fish Passage Center 1988). The cortisol and glucose responses of the second control group when presented with a prerelease handling-stress challenge indicated an unusual condition prior to release. Survival estimates of 103 and 108% for replicates one and three are possibly in error, but are considerably lower than the estimate of 131% for replicate two.

Test and control groups classified as descaled ranged from 0 to 3% (Table 3). A much larger percentage of the steelhead (60-77%) were classified as partially descaled, because they had one or both sides with > 3% of the area descaled. Comparison of prerelease samples and samples at release did not indicate crowding and loading significantly increased partial descaling. The number of partially descaled fish did not increase significantly with time as replicates 1-3 were released and did not differ between test and control groups (Table 3).

Smoltification: Gill ATPase activity levels of steelhead at Lyons Ferry SFH were low (mean = 9.4-11.7 umoles P_i mg protein^{-1} h^{-1}) among a11 groups at

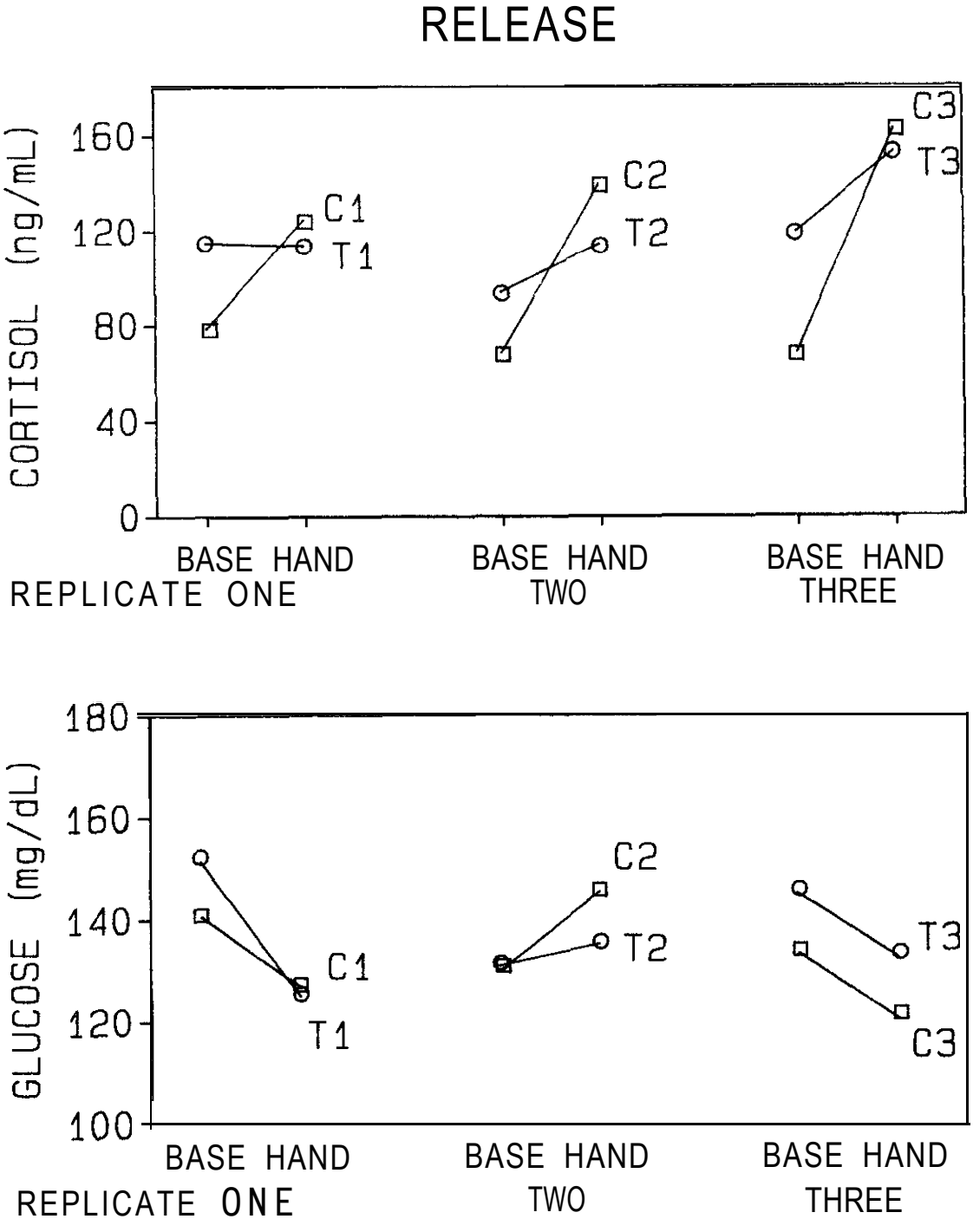

Figure 5. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile steelhead from Lyons Ferry SFH before (BASE) and one hour after a handling-stress challenge (HAND) performed at the time of release.

Table 3. Percent of juvenile steelhead from Lyons Ferry SFH classified as descaled and partially descaled in prerelease samples and at release.

Group	Prerelease			Release		
	Partial (%)	Descaled (%)	N	Partial (%)	Descaled (%)	N
Replicate 1						
Control	70	0	100	77	2	100
Test	61	1	100	72	0	100
Replicate 2						
Control	69	3	100	73	1	100
Test	63	1	100	60	0	100
Replicate 3						
Control	77	1	100	62	0	100
Test	77	2	100	65	2	100

the hatchery compared to levels subsequently measured at McNary Dam (mean = 9.1-35.2 umoles P_i mg protein $^{-1}$ h^{-1}; Figure 6). Mean ATPase levels at the hatchery were not significantly different between test and control groups of any of the three replicates and did not increase significantly at release from the first replicate to the third replicate (Appendix 3).

Mean ATPase levels of steelhead in control groups originating from Lyons Ferry were significantly lower than test groups when recaptured at McNary Dam (Figure 6; Appendix 3). As the migration at McNary Dam progressed through early, middle, and late (approximately 25, 50, and 75th percentile) the gill ATPase levels increased significantly in the test and control groups. The fish of the control groups collected early in the migration consistently had the lowest ATPase levels of a11 fish collected. Gill ATPase activities were higher in fish that had experienced more days of inriver migration (Figure 7A).

Mean plasma thyroxine levels of the third steelhead control group were significantly higher than levels in the third test group at Lyons Ferry SFH and at McNary Dam (Figure 7B; Appendix 4). Although mean thyroxine levels of control groups appeared to be slightly higher than means of test groups at McNary Dam, the difference was significant only in the third replicate. Mean plasma thyroxine levels increased significantly in all groups between levels at the hatchery and at McNary Dam. A post-release surge in thyroxine levels was indicated by elevated levels of thyroxine in control groups captured 3-14 days after release compared to levels at 8-22 days for test fish. An increase in thyroxine prior to increases in ATPase activity has been observed in other studies (Dickhoff et al. 1985).

Steelhead released from Lyons Ferry SFH did not have a silvery color, an appearance often associated with smoltification that appears about the time gill ATPase and plasma thyroxine activities increase. The color of steelhead in test and control groups ranged from mottled grey to nearly black compared to the silver color of the steelhead held in the production ponds at Lyons Ferry SFH.

Prior to release, fork lengths of test and control fish in each replicate were not significantly different (Figure 8; Appendix 5). Fish of the second replicate were slightly smaller than those in replicate one and three. In multivariate analysis, morphology of test and

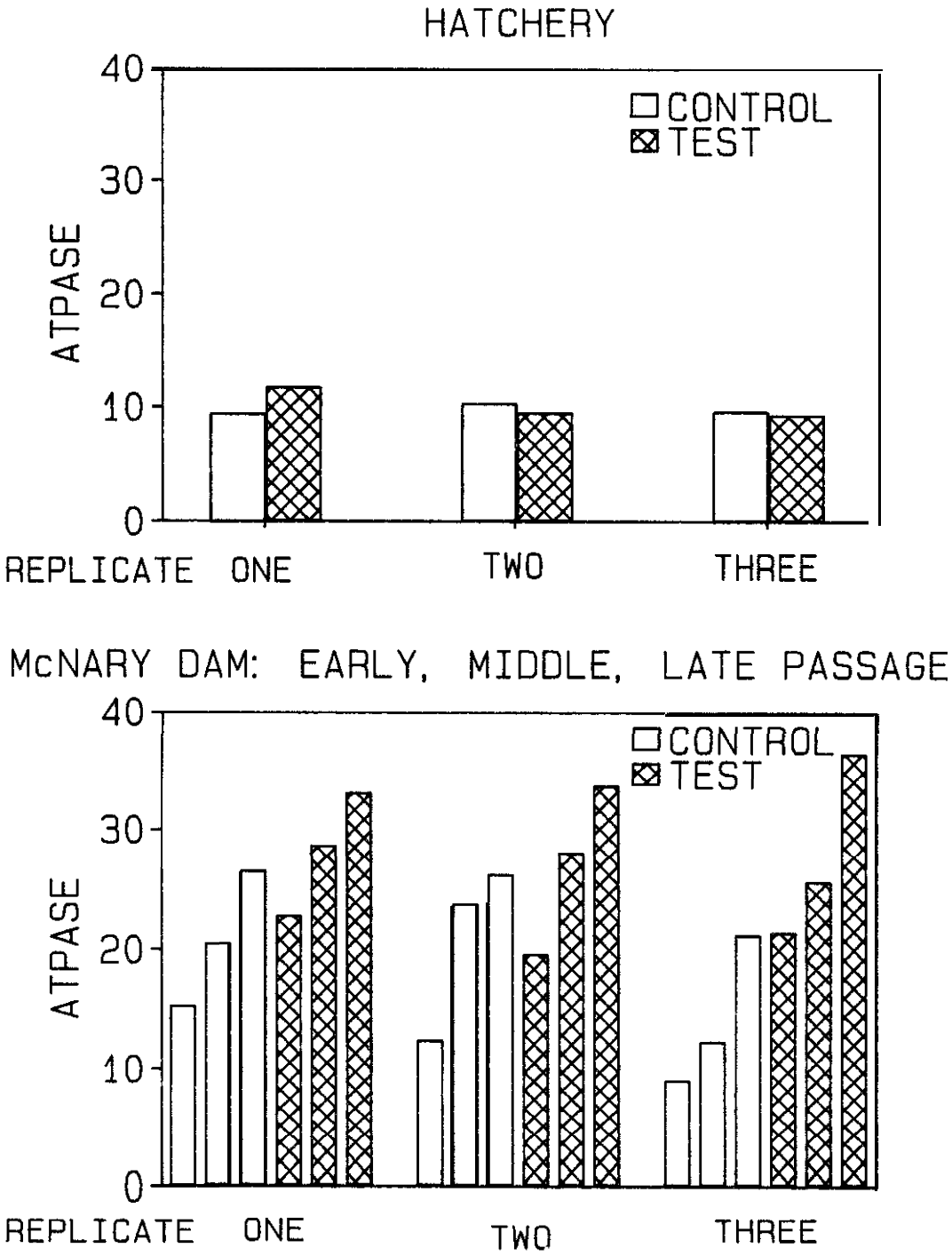

Figure 6. Mean gill $Na^{+}K^{+}$-ATpase activity (umoles P_i •mg protein-' •h^{-1}) levels of juvenile steelhead at Lyons Ferry SFH and in early, middle, and late segments of migration as the groups passed McNary Dam.

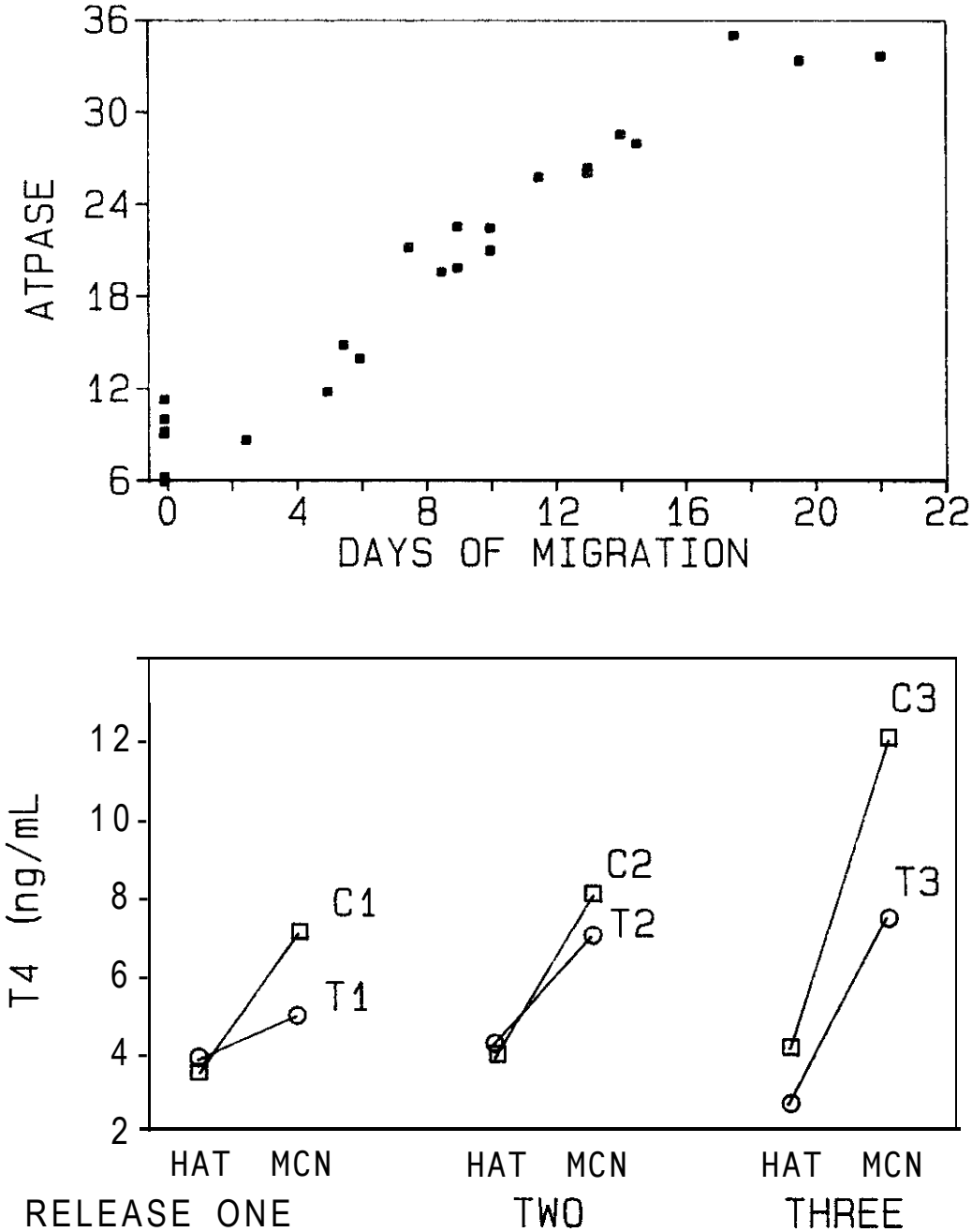

Figure 7. (A) Mean gill $Na^{+}K^{+}$-ATPase activity (umoles P_i•mg protein^{-1}•h^{-1}) levels of juvenile steelhead from Lyons Ferry SFH and days of inriver migration. (B) Mean plasma thyroxine (ng•mL^{-1}) levels of juvenile steelhead at Lyons Ferry SFH (HAT) and at McNary Dam (MCN). Samples collected at McNary Dam include 10 fish from the early, middle, and late segments of the migration (N = 30).

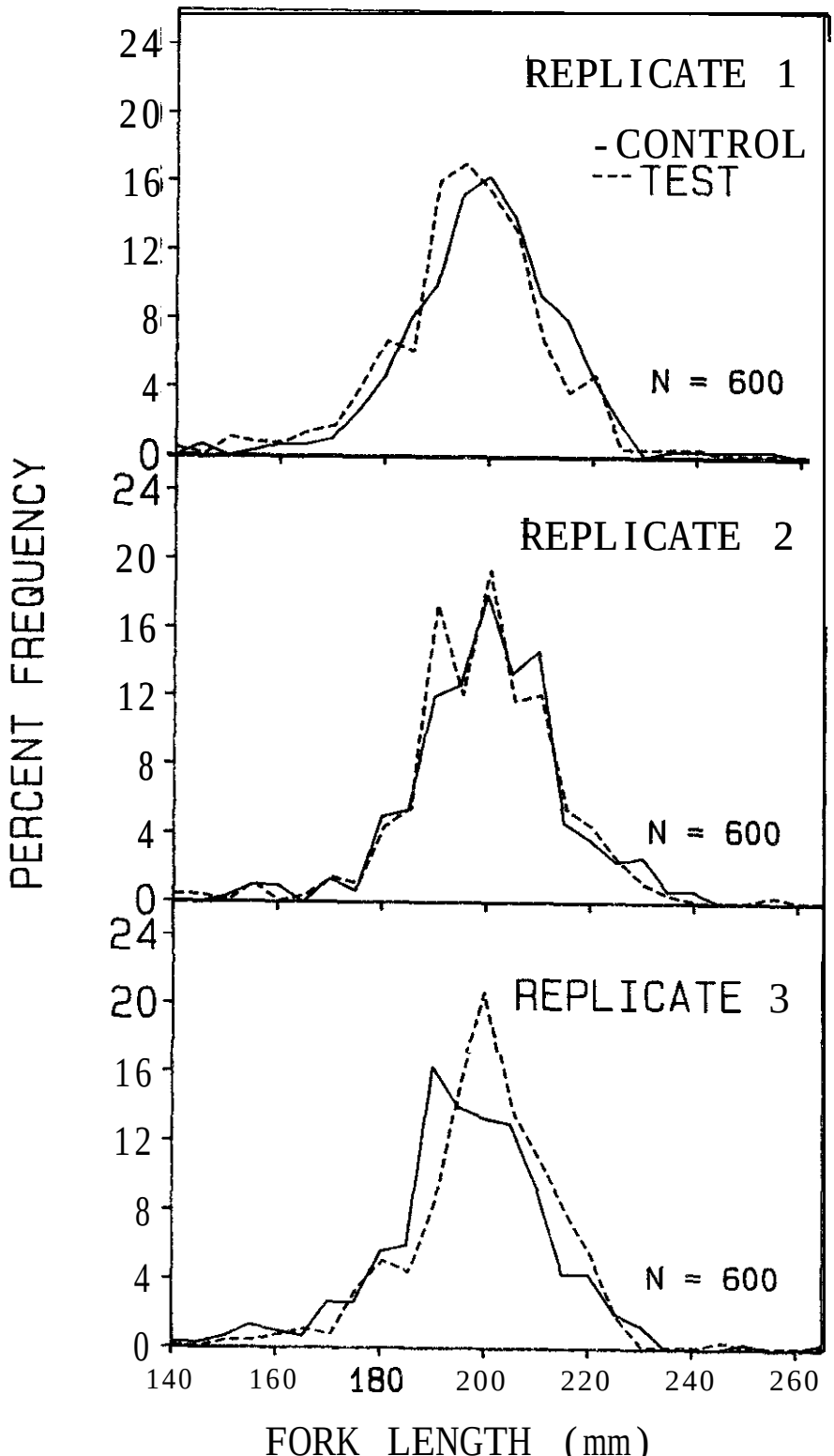

Figure 8. Length frequency distribution of juvenile steelhead sampled at Lyons Ferry SFH. Lengths are from fish sampled for stress, descaling, and smoltification indicators prior to release.

control groups Of Lyons Ferry steelhead was siqnificantly different in each replicate at McNary Dam. Univariate ANOVA indicated that the measurements contributing most to the differences in morphology were in the area of the head along with some measurements of body depth or caudal peduncle. None of the length measurements, from the anterior of the snout to various fins or to the posterior of the body, contributed to the differences.

After seawater challenges, blood ion levels of Lyons Ferry control groups were not significantly higher than those of corresponding test groups collected at McNary Dam (Table 4). Control groups were expected to have higher blood ion levels than test groups if controls had not yet developed osmorequlatory capacity, a part of smoltification. However, the early sample of test fish in the first replicate had significantly higher mean Na^+, Cl-, and osmolarity than control fish or a later sample of test fish (SNK; Table 4). The test sample with higher ion levels was unique in that it was one of only three in 18 groups that experienced any mortality during the seawater challenge; two fish (10%) died during the 24-h challenge. Four of the 18 survivors had physical irregularities, such as open abrasions, fungus on fins, or were thin and descaled. Fish with physical irregularities had Na^+ ranging from 185 to 264 $mmol \cdot L^{-1}$ while the remaining sample had a mean Na^+ of 185^+ 19 $mmol \cdot L^{-1}$ (mean, SD).

The control groups were different from the test groups in that plasma ion concentrations were often correlated with gill ATPase in samples from control groups and seldom in samples from the test groups. Plasma Na^+, Cl^-, and osmolarity were negatively correlated with gill ATPase in 6 of 9 data sets (range r = -0.41 to -0.61) of control groups from Lyons Ferry SFH, but for only one of 18 data sets in the test groups. These correlations reflect the relatively high ion levels observed among control fish with the lowest gill ATPase levels after a 24-h seawater challenge.

Steelhead from Wells SFH

Stress and descaling: At the time of release, plasma cortisol levels in steelhead from Wells SFH were significantly higher in test than in control groups of the second and third replicates (Figure 9; Appendix 6). Test groups differed from controls in that they were

Table 4. Mean plasma Na^+, Cl-, K+ ($mmol \cdot L^{-1}$), osmolarity ($mmol \cdot kg^{-1}$), and qill Na^+K^+-ATPase activity (umoles $P_i \cdot mg\ protein^{-1} \cdot h^{-1}$) of juvenile steelhead from Lyons Ferry SFH collected at McNary Dam and exposed to a 24-h seawater challenqe.

Group		Na^+	K+	CL^-	Osmolarity	ATPase
Replicate 1						
Control	1	169.6	5.0	153.5	335.5	21.4
Test	1-1	191.4	4.5	167.0	357.8	27.7
Test	1-2	172.2	5.1	159.3	336.2	30.9
Replicate 2						
Control	2	175.6	3.6	156.5	342.6	20.6
Test	2-1	164.1	4.1	149.8	322.7	30.4
Test	2-2	173.3	4.2	157.5	337.9	39.1
Replicate 3						
Control	3	168.9	4.8	155.0	334.0	19.5
Test	3-1	171.6	5.7	157.5	337.6	29.3
Test	3-2	172.4	4.4	158.4	339.4	37.7

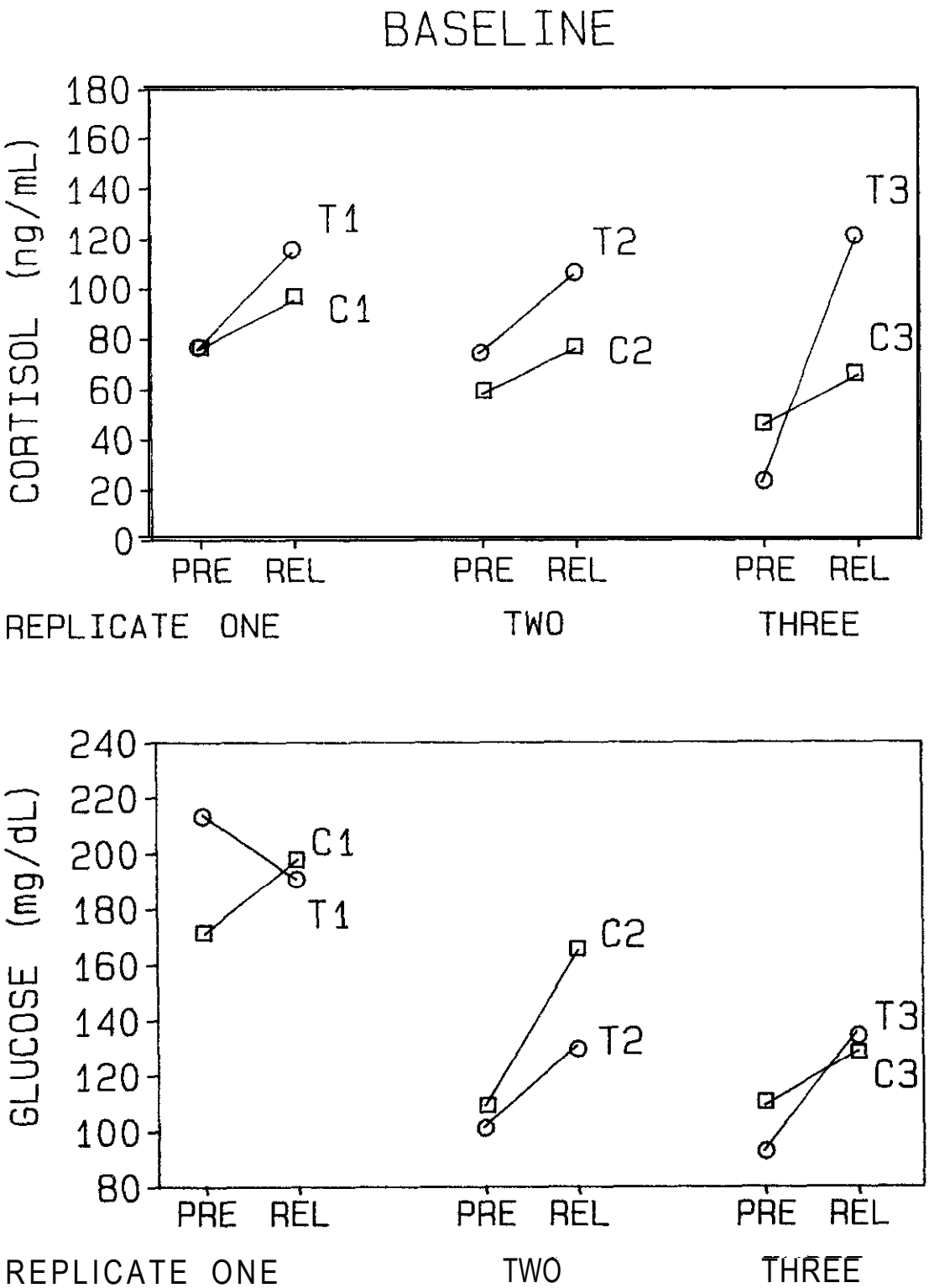

Figure 9. Mean baseline levels of plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) of juvenile steelhead in prerelease (PRE) samples collected at Wells SFH and in samples collected at release (REL) sites.

trucked for about 0.5 h at a density of 0.8 $lb \cdot gal^{-1}$ and controls were trucked for 2.5 h at 0.5 $lb \cdot gal^{-1}$.

Plasma cortisol levels were significantly higher at release than in prerelease samples in all three replicates (Figure 9; Appendix 6). I" addition, cortisol increase in the third test group was much greater than in any other group. That response was associated with a malfunction in the truck resulting in apparently hypoxic conditions and mortality of about 30% of the 12,000 fish in that truck.

Basal levels of plasma glucose in steelhead of the first replicate from Wells SFH were about two times the levels in prerelease samples from the other replicates (Figure 9). Prelease hyperglycemia observed among steelhead in the first replicate did not change significantly with the additional stress associated with release. Test and control groups of the second and third replicate had a normal response to the release procedure resulting in higher plasma glucose levels at release compared to basal prerelease levels.

In addition to being exceptionally high, plasma glucose levels in individual fish from the first replicate were highly variable resulting in coefficients of variation ranging from 38% to 84% compared to coefficients < 40% in other releases (Appendix 7). The high variability indicated the presence of some highly stressed fish in this replicate. These fish had only about 12 h recovery after completion of marking prior to loading and transportation to release sites, whereas fish of the other replicates had about 24 h for recovery. The erroneous survival estimates of replicate one (126%) may be associated with severe hyperglycemia. Survival estimates were 83 and 88% for the second and third replicates, respectively (Fish passage center 1988).

Steelhead from Wells SFH responded to the handlinq-stress challenge test with increases to similar levels of cortisol in prerelease and release samples (range 115.4 to 158.5 $ng \cdot mL^{-1}$; Figure 10 and 11). When presented with a handling-stress challenge at release, the increase in cortisol among control groups was larger (51.6 to 80.3 $ng \cdot mL^{-1}$) than the response in the test groups (10 to 29.1 $ng \cdot mL^{-1}$) (Figure 11). The larger response to the challenge at release among control groups can be attributed to their greater scope for response due to relatively low basal levels compared to fish in test groups.

Plasma glucose did not change significantly in

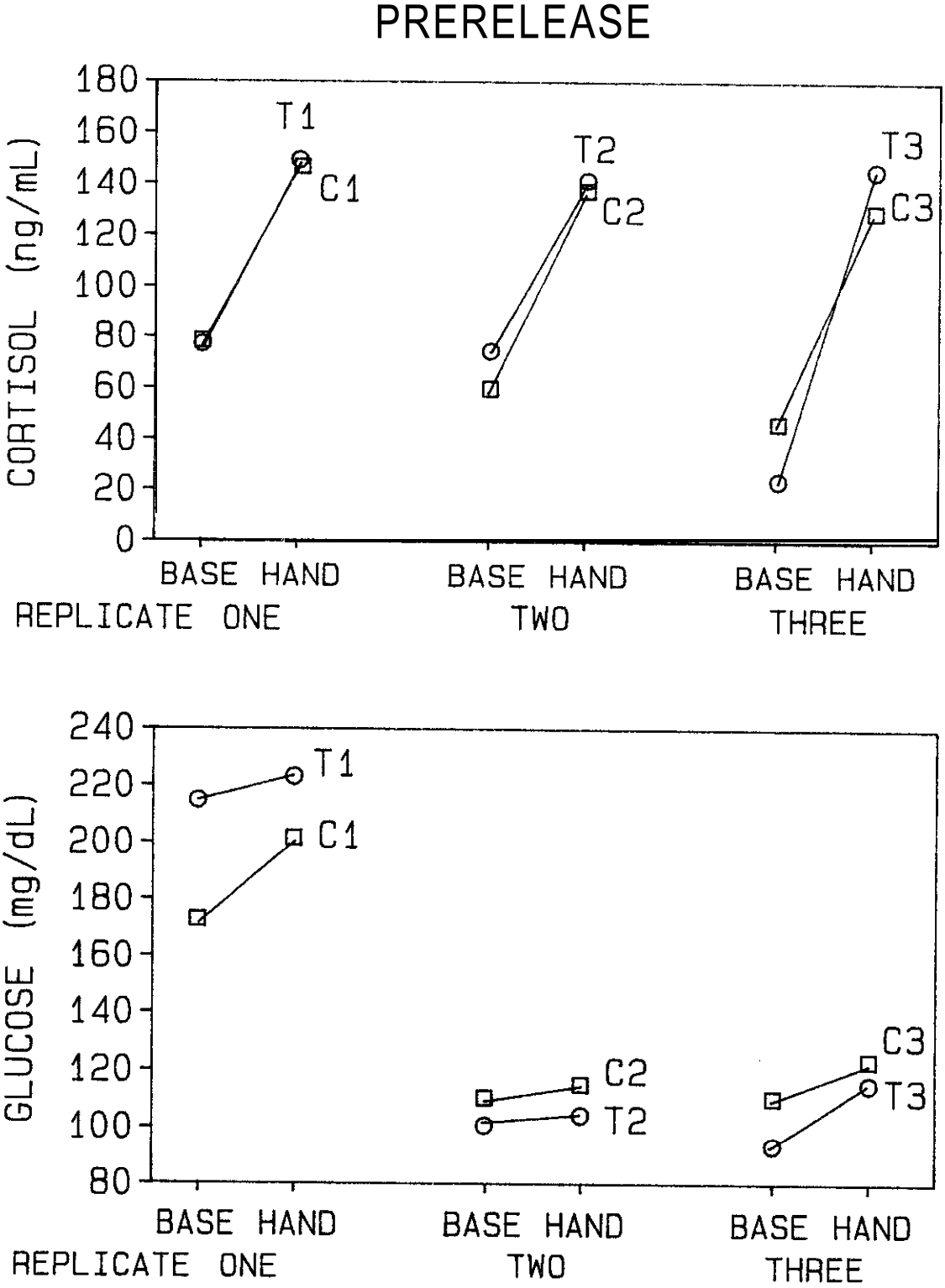

Figure 10. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile steelhead from Wells SFH before (BASE) and one hour after prerelease handling-stress challenge (HAND) performed prior to loading at the hatchery.

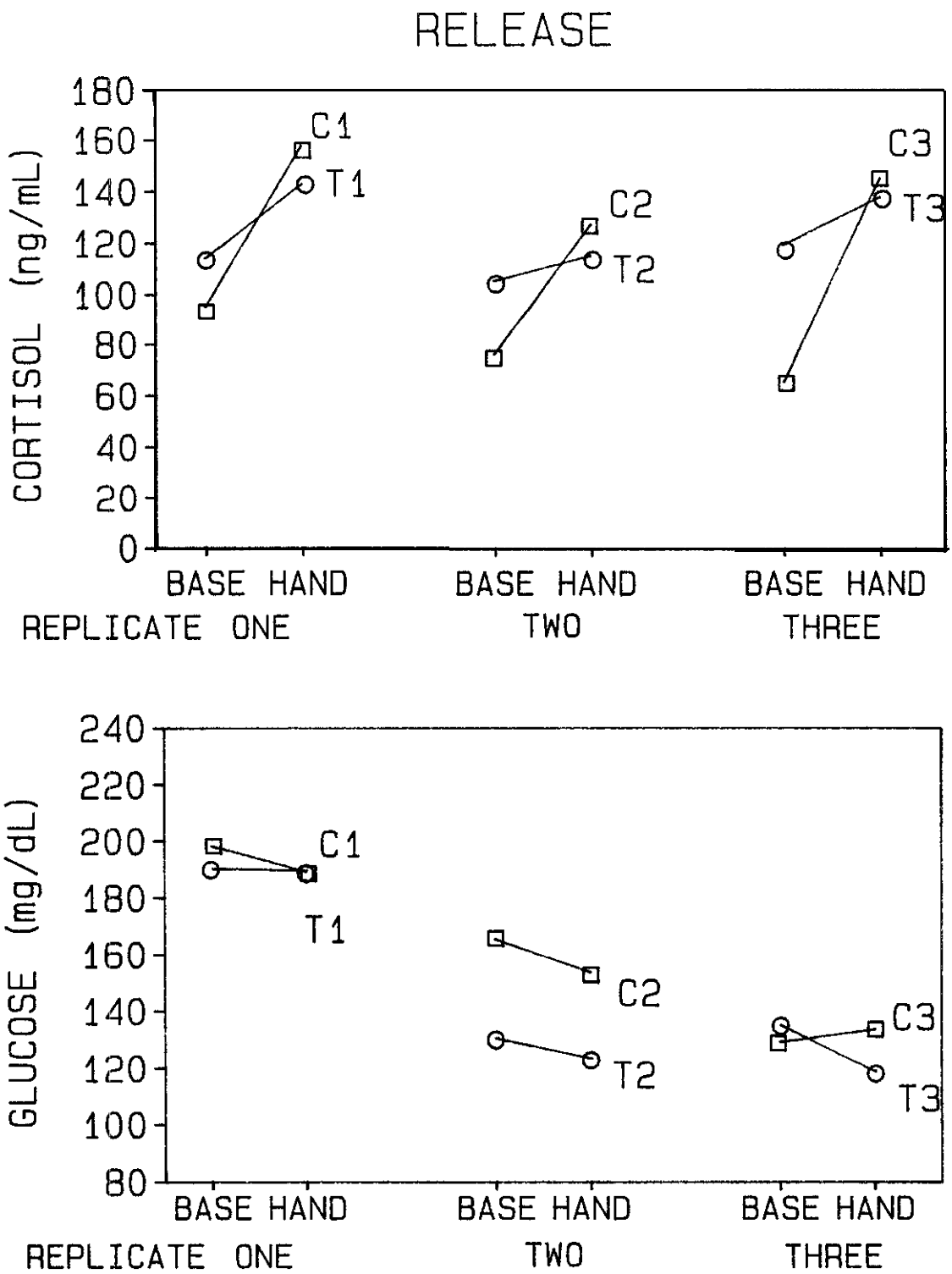

Figure 11. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile steelhead from wells SFH before (BASE) and one hour after a handling-stress challenge (HAND) performed at the time of release.

response to the handling-stress challenge in groups of any replicate (Figure 10 and 11; Appendix 7). Basal levels of glucose were exceptionally high in the first group and remained so after the challenge.

The percentage of test and control groups classified as descaled ranged from 0 to 2%, and partially descaled (one or both sides with > 3% of area descaled) from 37 to 79% (Table 5). Possible differences between test and control groups were not tested because of apparent observer bias. However, comparisons of the number of steelhead classified as partially descaled in each replicate indicated a significant increase with overall means of 52, 57, and 70% (N = 382, 400, and 400) for replicates 1-3, respectively.

Smoltification: Gill ATPase activity of steelhead at Wells SFH was not significantly different between test and control groups of each replicate. However, smoltification during the release period is evidenced by significantly increased gill ATPase as time passed between release of the first (April 23) and third (May 1) replicate (Figure 12; Appendix 8).

At McNary Dam, the ATPase activities of test and control groups were significantly different in the second and third replicates but not in the first (Figure 12; Appendix 8). In all test and control groups the ATPase activity increased significantly as the early, middle, and late (approximately 25th, 50th, and 75th percentile) segments of the migration passed McNary Dam. The highest levels of gill ATPaSe were found among fish that had experienced the most inriver migration time (Figure 13A).

When collected at McNary Dam, mean plasma thyroxine levels of Wells SFH steelhead in test and control groups were not significantly different (Figure 13R; Appendix 9). Thyroxine levels were significantly different between test and control groups at the hatchery. However, the relation of mean thyroxine in the test and control groups at the hatchery was different in replicate one compared to replicates two and three.

Morphology of steelhead from Wells SFH collected at McNary Dam was significantly different between fish of the test and control groups in the second and third replicates, but not different in the first replicate. Prior to release, mean fork length of test and control groups were not significantly different (Figure 14;

Table 5. Percent of juvenile steelhead from Wells SFH classified as descaled and partially descaled in prerelease samples and at release.

	Prerelease			Release		
Group	Partial (%)	Descaled (%)	N	Partial (%)	Descaled (%)	N
Replicate 1						
Control	50	1	100	41	2	100
Test	52	0	100	68	0	82
Replicate 2						
Control	59	0	100	61	0	100
Test	37	0	100	70	2	100
Replicate 3						
Control	63	1	100	67	0	100
Test	70	1	100	79	1	100

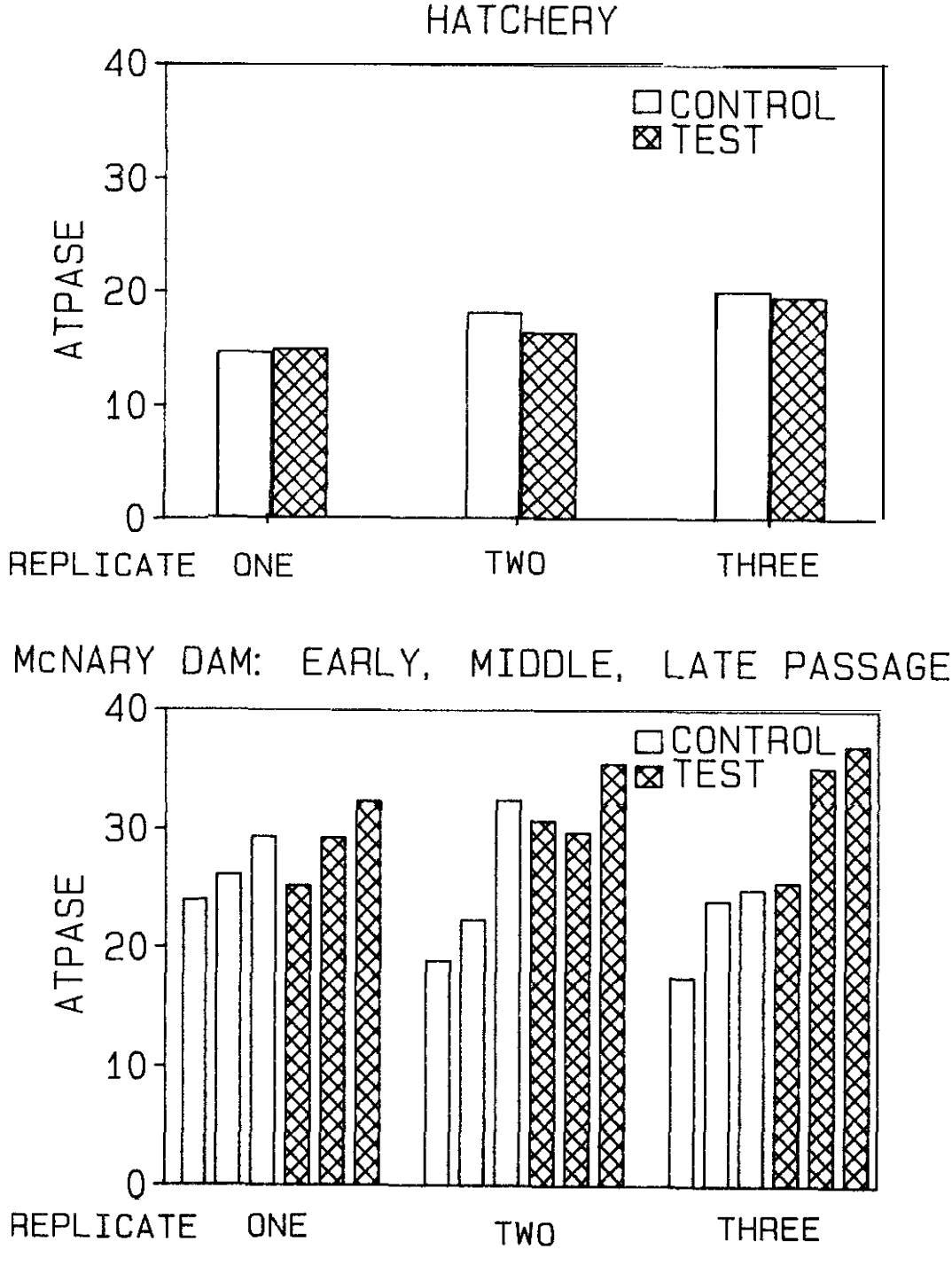

Figure 12. Mean gill $Na^{+}K^{+}$-ATPase activity (umoles P_i mg protein^{-1} h^{-1}) levels of juvenile steelhead at Wells SFH and in early, middle, and late segments of migration as the groups passed McNary Dam.

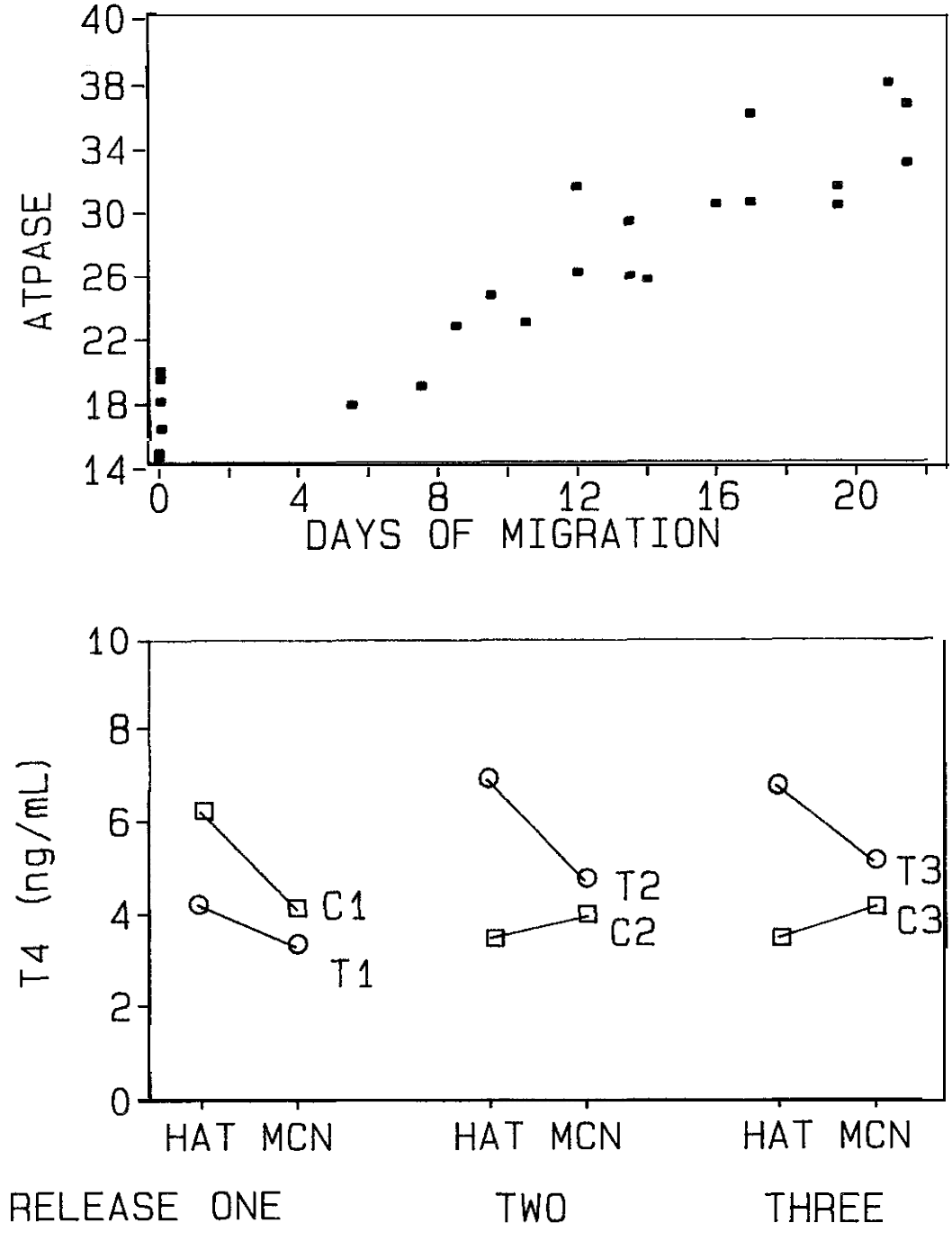

Figure 13. (A) Mean gill Na^+K^+-ATPase activity (umoles P_i •mg protein-' •h^{-1}) levels of juvenile steelhead at Wells SFH and days of inriver migration. (B) Mean plasma thyroxine (ng•mL^{-1}) levels of juvenile steelhead at wells SFH (HAT) and at McNary Dam (MCN). Samples collected at McNary Dam include 10 fish from the early, middle, and late segments of the migration (N = 30).

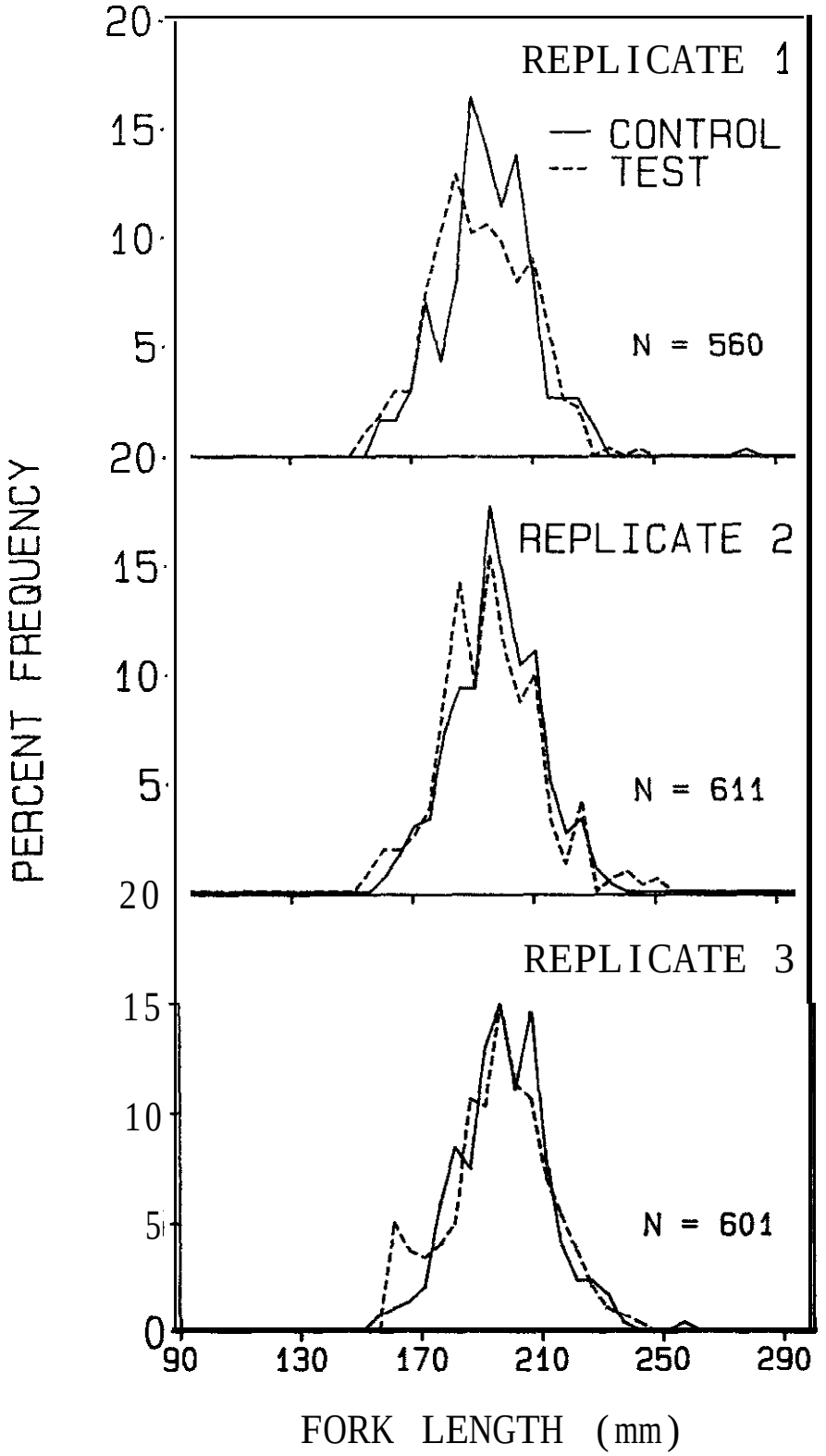

Figure 14. Length frequency distribution of juvenile steelhead sampled at Wells SFH. Lengths are from fish sampled for stress, descaling, and smoltification indicators prior to release.

Appendix 10). Univariate ANOVA indicated that measurements contributing to the morphological difference observed at McNary Dam in replicates two and three were from the snout to the origin of pectoral fin, from the posterior of dorsal fin to adipose fin, and total body length.

Steelhead in the control group from Wells SFH did not have significantly different plasma Na^+, Cl^-, and osmolarity than test groups after a 24-h seawater challenge (Table 6). Since differences in plasma ion levels were not observed we may conclude that their osmorequlatory ability, one characteristic of smoltification, was similar. However, samples from the control groups differed from test groups in that plasma Na^+, Cl^- and osmolarity were correlated with gill ATPase activity levels. Plasma Na^+, Cl^-, and osmolarity were negatively correlated with gill ATPase in 4 of 9 data sets (range $r = -0.47$ to -0.63) of control groups from Wells SFH, but in none of the 18 data sets for the test groups. The negative correlations reflect relatively high ion levels observed among fish with the lowest ATPase present in the control groups.

Spring Chinook from Winthrop NFH

Stress and descaling: At the time of release, plasma cortisol levels in juvenile spring chinook salmon from Winthrop NFH were significantly higher in the control groups than test groups of the first and second replicates (Figure 15; Appendix 11). Test groups were released directly from the hatchery raceway, therefore fish in the test groups experienced relatively little stress compared to control groups. In contrast, chinook salmon in control groups were trucked about 3.5h to their release site below Priest Rapids Dam. The cortisol levels observed in chinook salmon of the third test group were unlike replicates one and two and indicate an unexplained stressful condition prior to the start of the release procedure.

Mean plasma glucose levels of chinook salmon in the third control group were unusually high when released from the truck (mean = 171 $mg \cdot dL^{-1}$); glucose levels of control groups in replicates one and two were much lower, mean = 73, 84 $mg \cdot dL^{-1}$, respectively (Figure 8; Appendix 12). The fish in the third control group were held for about 0.5 h in a crowded condition just prior to loading on the truck, while a publicity film crew

Table 6. Mean plasma Na^+, Cl-, K+ ($mmol \cdot L^{-1}$), osmolarity ($mmol \cdot kg^{-1}$), and gill Na^+K^+-ATPase activity (umoles $P_i \cdot mg\ protein^{-1} \cdot h^{-1}$) of juvenile steelhead from Wells SFH collected at McNary Dam and exposed to a 24-h seawater challenge.

Group		Na^+	K+	CL^-	Osmolarity	ATPase
Replicate 1						
Control	1	169.7	5.2	151.3	324.6	25.2
Test	1-1	171.9	4.8	160.4	339.2	40.3
Test	1-2	171.2	4.4	158.7	335.0	37.1
Replicate 2						
Control	2	173.8	3.5	156.5	336.6	22.6
Test	2-1	173.2	5.3	157.7	336.7	38.4
Test	2-2	170.7	5.0	160.9	337.8	42.5
Replicate 3						
Control	3	169.0	5.5	156.5	330.3	28.4
Test	3-1	181.4	5.0	170.8	356.8	37.9
Test	3-2	177.4	3.3	164.3	343.6	44.3

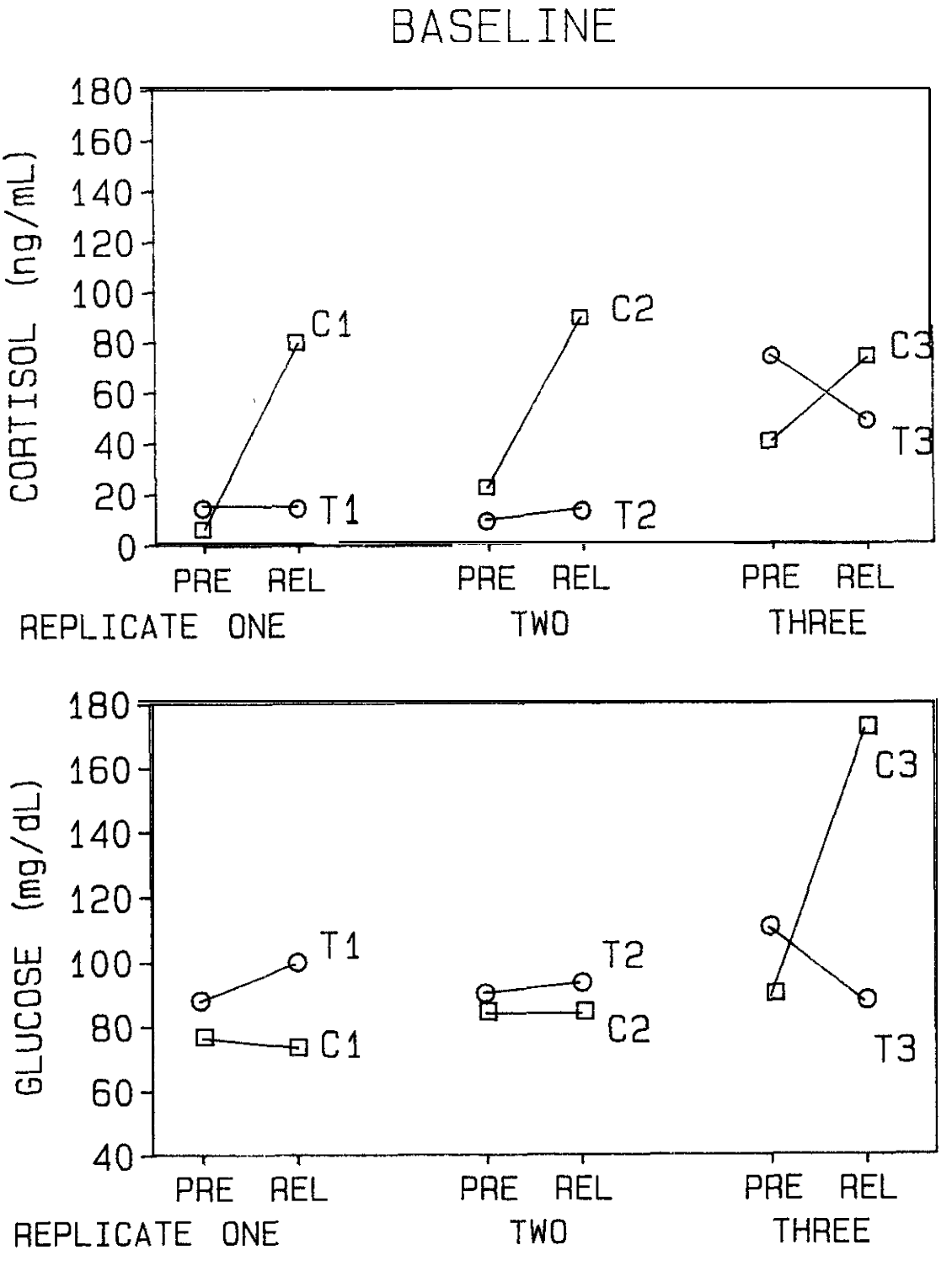

Figure 15. Mean baseline levels of plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) of juvenile spring chinook salmon in prerelease (PRE) samples collected at Winthrop NFH and in samples collected at the time of release (REL).

prepared to record the procedure. The approximate 4 h interval between the crowding prior to loading and subsequent sample at release probably allowed for a nearly maximal response in plasma glucose.

In both prerelease and release samples the response to the handling-stress challenge was a significant increase of plasma cortisol levels (Figure 16 and 17; Appendix 11). The results of the challenge in replicate one were not presented (Figures 16 and 17) because the protocol used was not correct.

Plasma glucose decreased dramatically in response to the handling-stress challenge at the release of the third control group (Figure 17). The glucose response of the third control group was significantly different from the response of the third test group. The third control group was subjected to about 0.5 h of crowding during publicity filming of the loading procedure and may not have been capable of maintaining osmoregulatory function after further stress from the challenge.

The pattern of glucose and cortisol levels in chinook of the third replicate were unlike those observed in the first and second replicate. Apparently, deviate stress indicators of the third replicate did not affect survival estimates. In this case, the survival estimates were 49, 61, and 33% for replicates 1-3, respectively. The stress responses of the control group of the second replicate were not unusual.

The percentage of test and control groups classified as descaled ranged from 0 to 4% (Table 7). The percentage of chinook salmon classified as partially descaled (one or both sides with > 3% of area descaled) ranged from 41% to 92%. The number of chinook salmon classified as partially descaled increased significantly from an overall average of 63% (N = 391) in the second replicate to 82% (N = 399) in the third replicate.

Smoltification: Mean gill ATPase activity levels of juvenile chinook salmon in the test and control groups were not significantly different at Winthrop NFH (Figure 18; Appendix 13). ATPase did not change from the date of the release of the first (April 20) to the third (April 28) replicate.

Mean ATPase activity levels of chinook salmon in the test and control groups were significantly different when collected at McNary Dam during the migration (Figure 18; Appendix 13). The pattern of increasing ATPase levels observed among steelhead as the migration progressed through early, middle, and late segments was

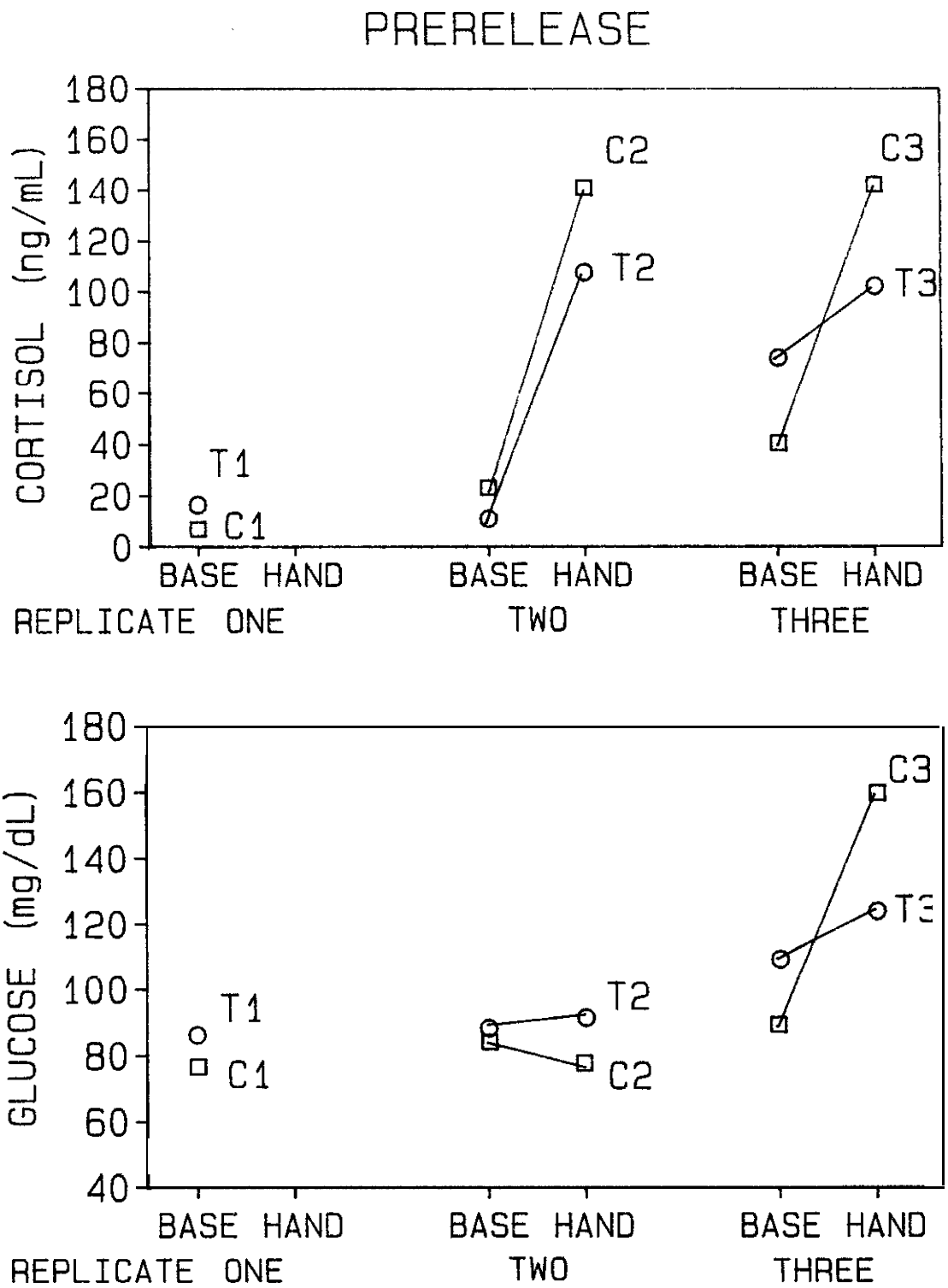

Figure 16. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile spring chinook salmon from Winthrop NFH before (BASE) and one hour after a prerelease handling-stress challenge (HAND) performed prior to release procedures.

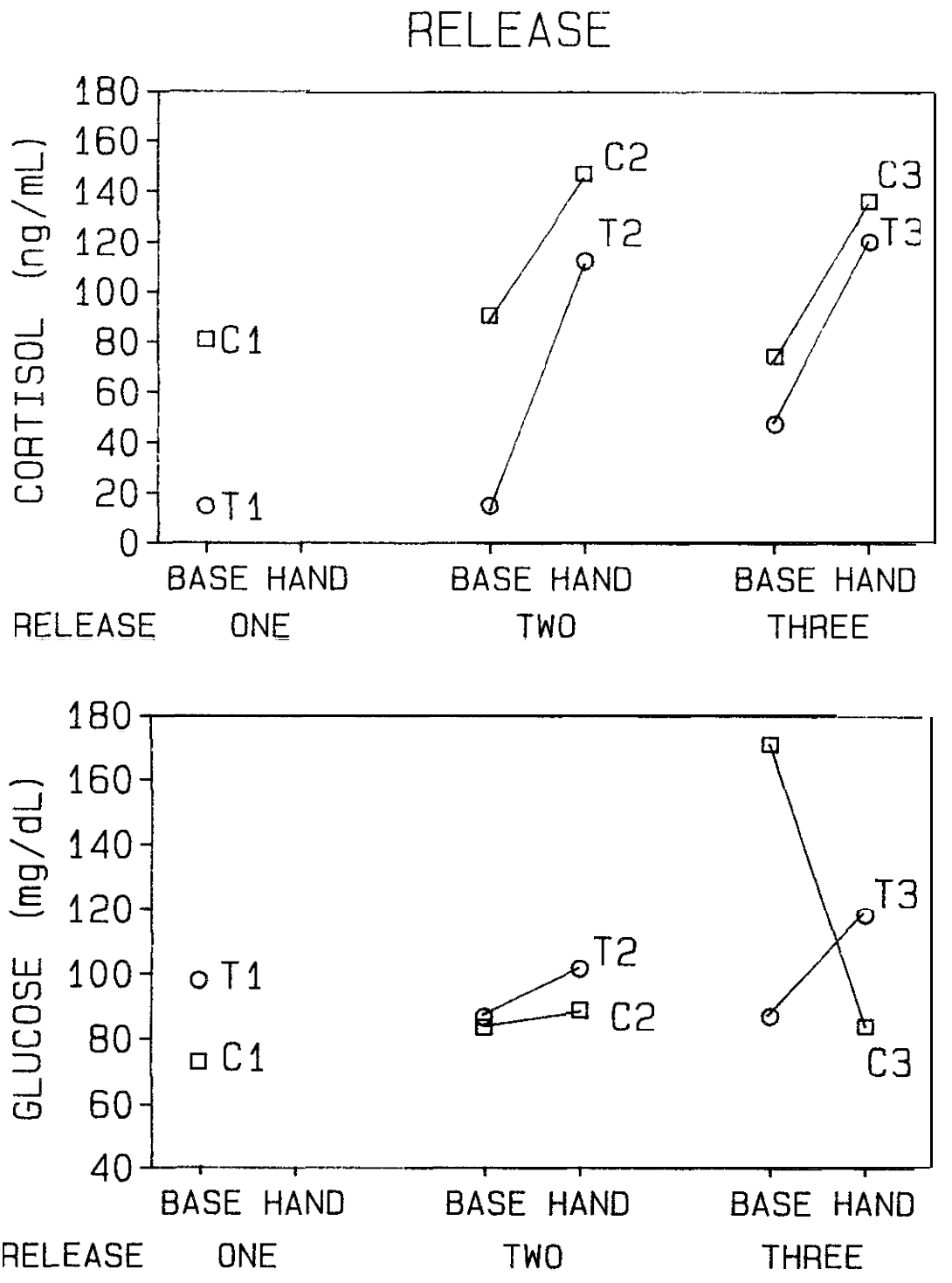

Figure 17. Mean plasma cortisol ($ng \cdot mL^{-1}$) and glucose ($mg \cdot dL^{-1}$) levels of juvenile spring chinook salmon from Winthrop NFH before (BASE) and one hour after a handling-stress challenge (HAND) performed at the time of release.

Table 7. Percent of juvenile spring chinook salmon from Winthrop NFH classified as descaled and partially descaled in prerelease samples and at release.

Group	Prerelease			Release		
	Partial (%)	Descaled (%)	N	Partial (%)	Descaled (%)	N
Replicate 1						
Control	-	0	100		4	96
Test		1	100		0	100
Replicate 2						
Control	72	1	100	41	2	91
Test	58	1	100	78	4	100
Replicate 3						
Control	92	4	100	63	0	100
Test	87	1	99	87	0	100

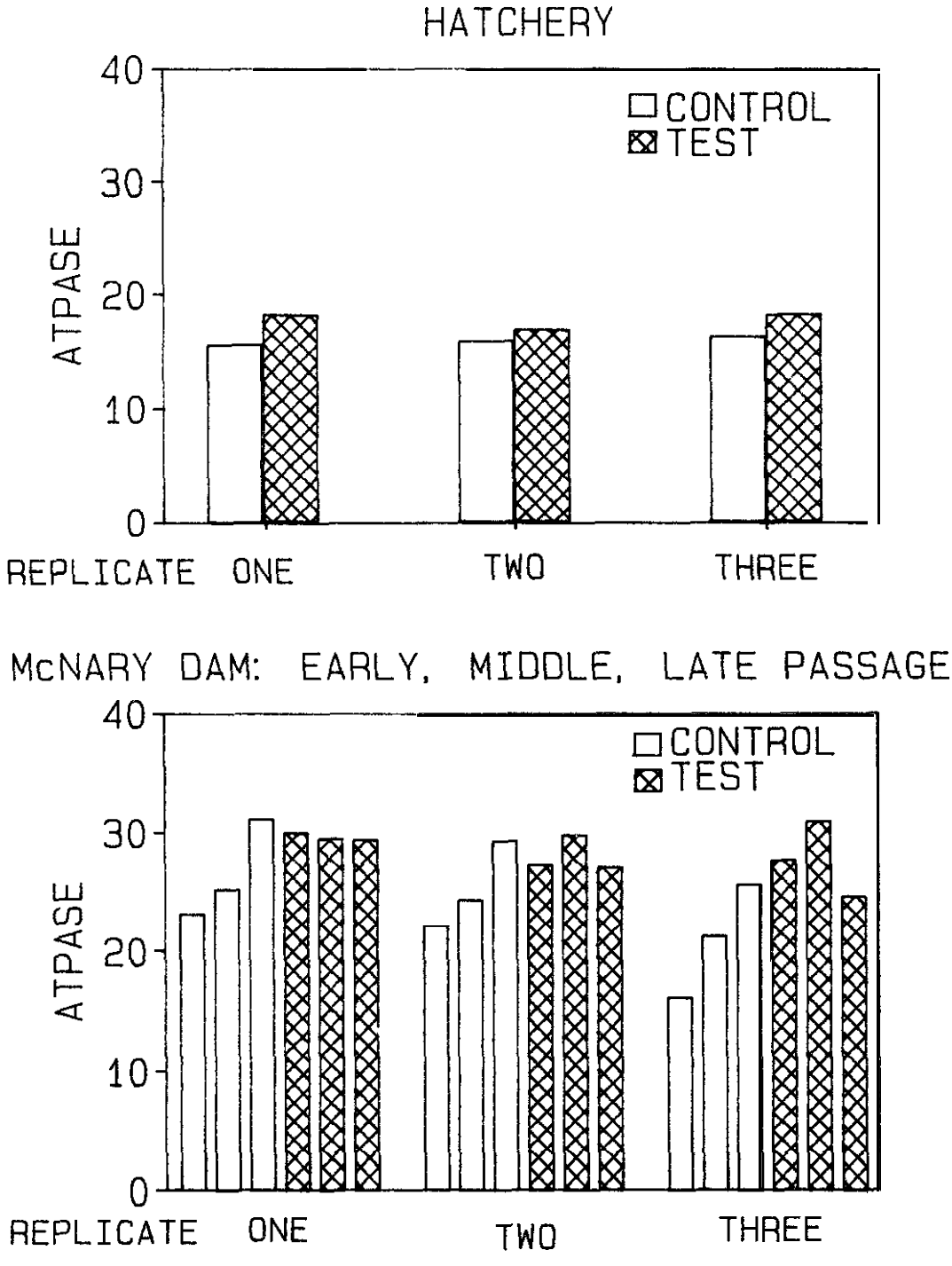

Figure 18. Mean gill $Na^{+}K^{+}$-ATPase activity (umoles P_i • mg $protein^{-1} \cdot h^{-1}$) levels of juvenile spring chinook salmon from Winthrop NFH and in early, middle, and late segments of miqration as the groups passed McNary Dam.

evident in the control groups (Figure 18), but not in the test groups (statistical interaction, Appendix 13b). Mean gill ATPase increased progressively during the first 20 days of inriver migration (Figure 19A).

Mean plasma thyroxine levels of chinook salmon in the test and control groups of the first and second replicates were significantly different at McNary Dam (Figure 19B; Appendix 14). The difference was greatest in the second replicate where the test group had a mean level of 11.2 $ng \cdot mL^{-1}$ and the control group 4.8 $ng \cdot mL^{-1}$. The overall mean thyroxine level of fish sampled at the hatchery increased significantly to 3.3, 4.4, and 6.3 $ng \cdot mL^{-1}$ for replicates 1-3, respectively.

Morphology of chinook salmon from Winthrop NFH was significantly different between fish of the test and control groups in each replicate collected at McNary Dam. Significant differences in morphology can be attributed primarily to differences in size (a factor in the analysis) between test and control fish as they migrated past McNary Dam. Univariate ANOVA indicated almost all of the 26 measurements made on the fish of test and control were significantly different.

Test and control fish of each replicate were also significantly different in size when released (Figure 20; Appendix 15). The difference apparently developed because the control groups were held on a restricted ration to slow growth. Experience in prior years had shown that if the control fish were held at about 12,000 fish per raceway and test fish at about 20,000 fish per raceway the lower density of the control fish often resulted in larger fish.

BKD in Chinook salmon:

Results of the FAT and ELISA methods for detection of BKD did not indicate the same prevalence of disease; FAT was less sensitive in detecting the disease. Prevalence of a disease is its frequency at a given moment in time (MacMahon et. al 1960). Using the FAT, prevalence of BKD was not significantly different among groups tested at Winthrop NFH and at McNary Dam (Table 8). The ELISA method identified a greater percentage of BKD positive fish than the FAT method, and indicated the prevalence of BKD differed between the test and control groups.

According to ELISA tests, prevalence of BKD at release was significantly higher in test fish than in control fish (Table 9), presumably due to the higher

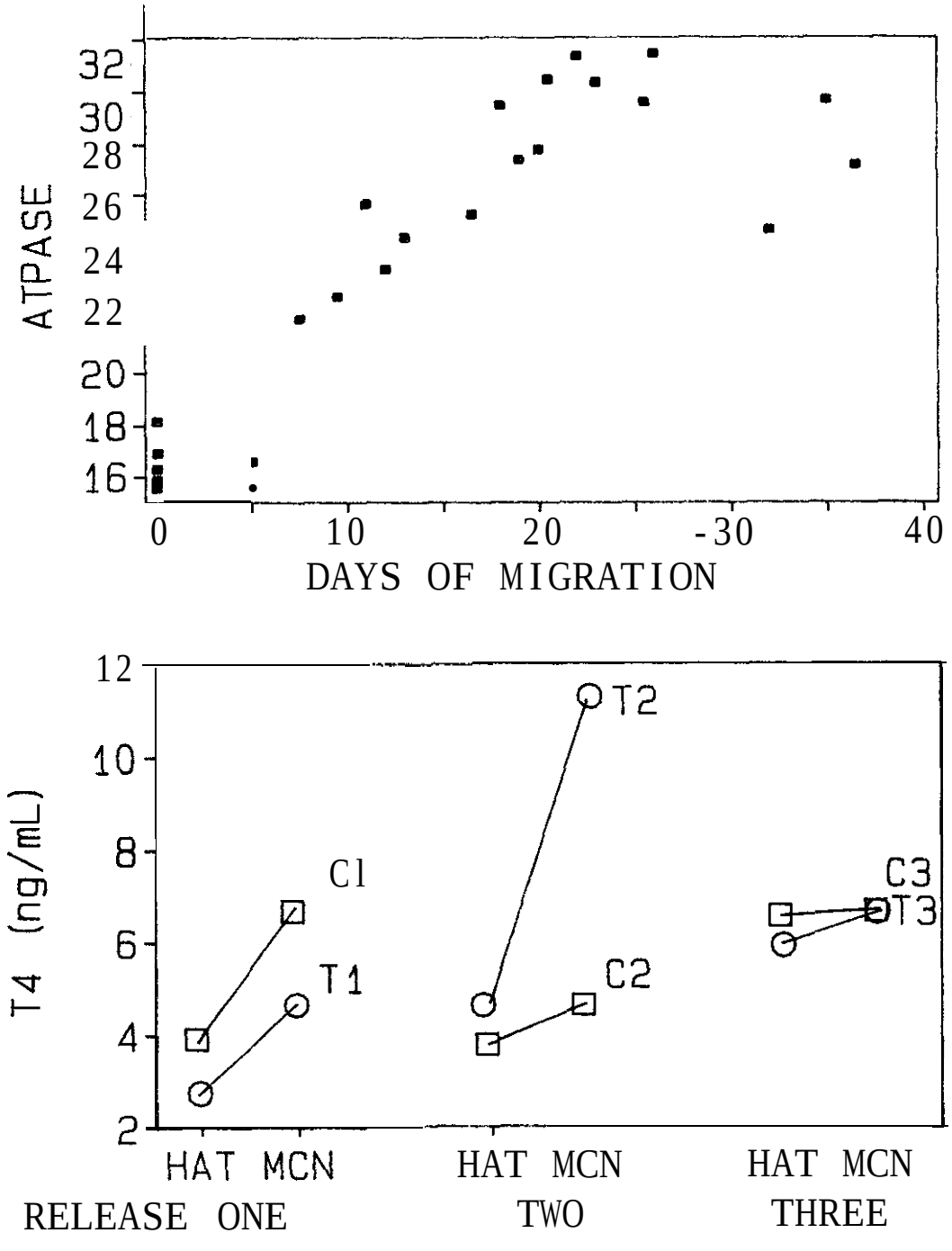

Figure 19. (A) Mean gill Na^+K^+-ATPase activity (umoles P_i•mg protein^{-1}•h^{-1}) levels of juvenile spring chinook salmon from Winthrop NFH and days of inriver migration. (B) Mean plasma thyroxine (ng•mL^{-1}) levels of juvenile spring chinook salmon at Winthrop NFH (HAT) and at McNary Dam (MCN). Samples collected at McNary Dam include 10 fish from the early, middle, and late segments of the migration (N = 30).

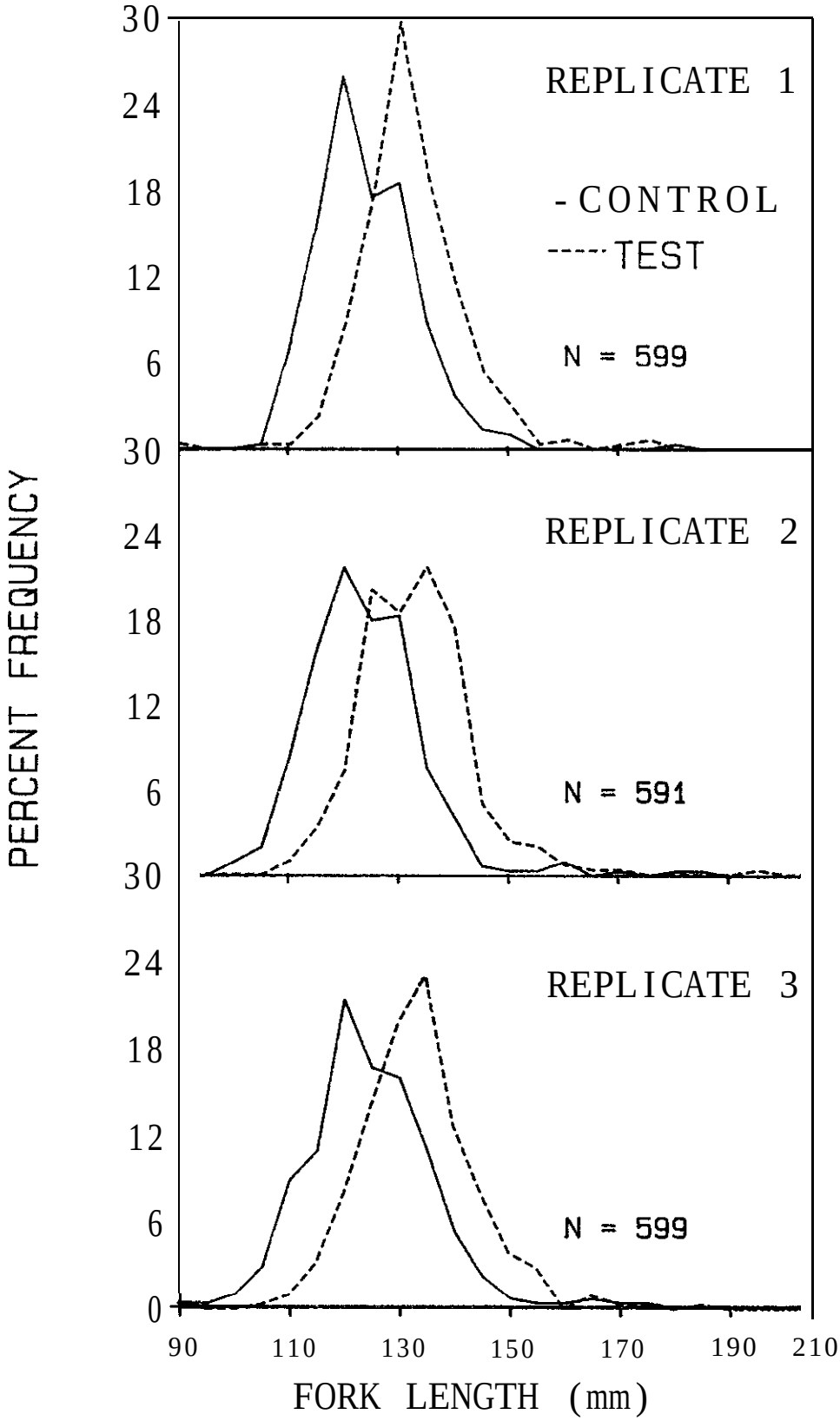

Figure 20. Length frequency distribution of juvenile spring chinook salmon sampled at Winthrop NFH. Lengths are from fish sampled for stress, descalinq, and smoltification indicators prior to release.

Table 8. Percent of juvenile spring chinook salmon from Winthrop NFH classified according to selected categories of bacteria counts in the fluorescent antibody test (FAT) for fish collected at the hatchery and in early, middle, and late segments of the migration past McNary Dam.

SAMPLE SITE	LEVEL			
	Negative	LOW	Medium	High
Winthrop NFH				
control	67.1	25.3	3.8	3.8
test	65.1	18.1	3.6	13.2
McNary Dam				
early	78.4	15.0	3.3	3.3
middle	78.3	15.0	1.7	5.0
late	69.0	22.6	2.8	5.6

Table 9. Percent of juvenile spring chinook salmon from Winthrop NFH ciassified according to selected categories of optical densities in the enzyme-linked immunosorbent assay (ELISA) of fish tissue collected at the hatchery and in early, middle, and late segments of the migration past McNary Dam.

SAMPLE SITE	LEVEL			
	Negative	LOW	Medium	High
Winthrop NFH				
control	65.8	5.1	13.9	15.2
test	43.4	16.9	14.4	25.3
McNary Dam				
early	80.0	10.0	3.3	6.7
middle	78.3	8.3	6.7	6.7
late	63.6	5.5	18.2	12.7

densities in the test raceways. I" addition, fish sampled from the test groups at the hatchery had a higher prevalence of BKD than the recapture population at McNary Dam. About 57% of the test fish were positive for BKD at Winthrop NFH and 26% of the recaptured population at McNary Dam.

Prevalence (percent) of BKD among migrants as determined by ELISA increased with time as early (20.0%), middle (21.1%), and late (36.6%) portions of the migration passed McNary Dam. Control fish were not sampled for BKD at McNary Dam. Travel times for the early, middle, and late samples from the migration were 20, 25, and 34 days, respectively.

Examination of recaptured chinook from each test group indicated visual signs, characteristic of acute BKD infection, in 1.7, 6.0, and 19.8% of the fish in the early, middle, and late segments of the migration, respectively. Visual signs of BKD, although not definitive, indicated a high preve1ence o f acutely infected (19.8%) fish late in the migration and this was supported by the ELISA results (36.6% positive). The visual signs were confirmed by ELISA inasmuch as when visual examination indicated no signs of BKD, the fish were negative 77% of the time and when such signs were present, 87% of the fish tested positive (Table 10).

Table 10. Percent of juvenile spring chinook salmon from Winthrop NFH classified as positive and negative for bacterial kidney disease (BKD) by visual examination, confirmed by the fluorescent antibody test (FAT) and enzyme-linked immunosorbent assay (ELISA) methods. Samples were from the hatchery and McNary Dam.

	WINTHROP NFH	
Clinical diagnosis	FAT confirmed	ELISA confirmed
Negative	71.1	58.4
Positive	92.3	92.3
	McNARY DAM	
Clinical diagnosis	FAT confirmed	ELISA confirmed
Negative	80.5	76.6
Positive	82.4	87.5

DISCUSSION

Stress and descaling:

Stress in steelhead released from Lyons Ferry and Wells SFH, as indicated by plasma cortisol, was generally higher among test fish than among the control group of the same replicate. This was probably due to the partial recovery of control groups from the stress of loading during the longer transportation. Alternatively, test fish may have experienced greater stress associated with crowding and loading larger numbers of fish, and from being transported at higher densities. Originally, we were concerned that control fish were being released in a more stressed condition than test fish, however, the results for releases from Lyons Ferry and Wells SFH indicate the opposite occurred.

Test groups of juvenile spring chinook salmon from Winthrop NFH were released directly from the hatchery raceway rather than being transported prior to release. Relatively low levels of stress in these groups of fish, as indicated by plasma cortisol, were thought to be the result of this release strategy.

Unexpected cortisol and glucose levels, or responses to the handling-stress challenge, occurred in at least one replicate from each hatchery. In several cases this was the result of changes in equipment or protocol. No association was identified between stress indicators and survival estimates for juvenile chinook from Winthrop NFH. Replicates from Wells SFH and Lyons Ferry SFH with deviate stress indicators also had unexpectedly high survival estimates. To further understand possible causitive mechanisms, the survival estimates must be examined in greater detail.

The survival estimate for the first replicate of steelhead from Wells SFH was considerably higher (126%) than estimates of replicates two (83%) and three (88%). The passage index proportions for control groups, a component of each survival estimate based on recovery of freeze branded steelhead, were 0.302, 0.366, and 0.332 for control groups 1-3, respectively (Fish Passage Center 1988). In addition, the passage index

proportions for test groups were 0.380, 0.304, and 0.292, respectively. Since the survival estimate is the ratio of test and control proportions, the relatively low index proportion for the first control group and high proportion for the test group contribute to the high estimate.

Severe hyperglycemia in test and control groups of steelhead of the first replicate was associated with the recovery time from marking and handling of about 12 h compared to about 24 for other groups. Even though glucose levels in this replicate were relatively hiqh, prerelease cortisol levels were at about the same level as levels of fish in replicate two. The 12 h recovery period may have been sufficient time for a normal increase. and a subsequent decrease in levels Of cortisol, but it afforded a nearly maximal response of glucose, if steelhead respond to multiple stressors similar to chinook salmon (Barton et al. 1986). Since control fish from replicate one were released without adequate time for recovery, they may have been less able to avoid predation than the other controls, resulting in the lower proportion of recovery. However, this scenario does not explain the relatively high proportion recovered for the first test group from Wells SFH which may have also contributed to the erroneous estimate.

A similar relation between the passage index proportions of control (0.306, 0.277, and 0.315) and test groups (0.315, 0.361, and 0.340) of the second replicate from Lyons Ferry steelhead resulted in a survival estimate of 131%. Glucose levels of steelhead in the control group of this replicate were unusually low after the prerelease handling-stress challenge, and unlike other replicates, test and control fish in the second replicate were able to maintain glucose levels at the time of release. These observations suggest qroups of the second replicate did not respond to loading and trucking in the same manner as others.

Steelhead in the second replicate were slightly smaller than the other tWO replicates. Considering this difference, as well as differing response observed during the handling-stress challenge, we speculate there may have been metabolic differences between replicates. Glucose metabolism is known to be sensitive to differences in feeding ration and parr-smolt transformation (Wedemeyer 1976; Woo et al. 1378). However, smoltification indicators did not show that fish in replicate two were more or less developed smolts.

Explanations for differences in glucose response may be advanced, however, the importance is that the assumption that fish of all replicates were similar was not met.

The following null hypothesis and reasons for the acceptance or rejection are listed for each hatchery.

Objective 1 Ho: Transportation and release of control fish is no more stressful than modes of releasing test fish.

Accept Ho: for steelhead from Lyons Ferry SFH. Basal plasma cortisol levels and response to the handling-stress challenge indicated that just the opposite occurred. Test groups may be more stressed and have less Scope for additional cortisol response compared to control groups.

Accept Ho: for steelhead from Wells SFH. The null hypothesis was accepted for the same reason as for steelhead from Lyons Ferry SFH.

Reject Ho: for chinook from Winthrop NFH. Juvenile chinook salmon in the control groups were transported for about 3.5 h and had a two to ten fold increase in cortisol levels compared to basal prerelease levels while fish of the test group exhibited no increases in response to the release procedures.

Smoltification: I" past years survival estimates for steelhead from Lyons Ferry SFH were over 100% (Fish Passaqe center 1988). The major difference between steelhead originating from Lyons Ferry SFH and from Wells SFH used in survival estimates during 1987 were related to smoltification. Those differences included: (1) low gill ATPase activity of steelhead at Lyons Ferry SFH, with no evidence of increase between first and third replicate releases; (2) steelhead from Lyons Ferry SFH had consistent differences among gill ATPase of early, middle and late segments of the migration of test and control fish; (3) a consistent increase in plasma thyroxine levels of steelhead between Lyons Ferry SFH and McNary Dam; and (4) Lyons Ferry SFH was the only source of fish with morphological differences between test and control groups in all replicates when size was not a factor.

Several factors may have contributed to the differences observed between fish from Lyons Ferry SFH and Wells SFH. First, holding the fish in the raceways

at Lyons Ferry SFH two to three weeks after marking apparently had a deleterious effect on condition, suppressing smoltification. Since production fish were not held in this manner these observations are not applicable to those fish. Second, control groups from Lyons Ferry SFH had travel times to McNary Dam ranging from 6 to 8 days compared to 8 to 12 days for steelhead from Wells SFH. During this migration time the progressive increases in gill ATPase activity indicates the steelhead were undergoing smoltification. Therefore, the longer the travel time to McNary Dam the more time for smolt development. This relationship results in control groups from Lyons Ferry SFH being least developed.

The test and control groups of the first replicate released from Wells SFH had the longest travel times of all steelhead groups sampled, and they exhibited no differences in smoltification indicators. Travel times of the first test and control groups were 17 days and 12 days, respectively. Travel times of other test and control groups of steelhead released from Wells SFH were 14 and 8 days, respectively. The additional inriver travel time for the first control group minimized the differences usually observed at McNary Dam between test and control groups.

Objective 2 Ho: Fish in test and control groups used to estimate survival are not at different stages of smoltification.

Reject Ho: for steelhead from Lyons Ferry SFH. The acceptance of the alternative hypothesis that the steelhead were at different stages of smoltification is supported by the data on gill ATPase, thyroxine, and morphology.

Reject Ho: for steelhead from Wells SFH. Rejection of the null hypothesis is supported for replicates two and three by differences in gill ATPase in freshwater, ATPase after seawater challenge, and morphology of test and control groups. The rejection of the ""11 hypothesis is not supported by data from the groups of the first replicate in which no differences existed for gill ATPase, thyroxine, morphology, or plasma ions after seawater challenge.

Reject Ho: for juvenile spring chinook salmon from Winthrop NFH. The acceptance of the alternative

hypothesis is supported by data on gill ATPase and possibly thyroxine. Perhaps more important is that the size of the test and control fish differed by about 10 mm at release and the morphological analysis indicated a difference still existed at McNary Dam. The data provides compelling evidence that the test and control groups of chinook salmon were not the same based simply on size.

BKD in Chinook Salmon:

Differences in the prevalence of BKD in test and control groups were evident using the ELISA, but not the FAT method. Differences in these methods demonstrated previously and experience has shown that ELISA is a more sensitive method of BKD detection. Therefore, further discussion will be based on results of the ELISA tests.

Three observations can be made from results of the ELISA data. First, prevalence of BKD was higher at Winthrop NFH than at McNary Dam suggesting a number of fish infected with BKD disappeared from the population between release from the hatchery and recapture at McNary. Second, more fish with acute infections were present in the late portion of the migration, indicating fish with acute infections migrate slower than those either not infected or infected to a lesser degree. An alternative explanation is that the longer travel time permitted the development of more acute symptoms observed in fish migrating at slower rates. And third, visual examinations that identified fish with acute infections of BKD were generally in agreement with results of the ELISA at McNary Dam. These findings did not support the possibility that lower prevalence of BKD at McNary was due to a post-release recovery from BKD during the migration.

Objective 3 Ho: The proportion of spring chinook salmon with chronic infections and high antigen levels for BKD is the same at release and at recapture downstream.

Reject Ho: Spring chinook salmon with chronic infections disappeared from the migrating population, presumably because of mortality.

RECOMMENDATIONS

1. We recommend that even small deviations from mark and release protocols be guarded against because they can result in unexpected conditions that can be stressful to the fish and can alter subsequent survival estimates.

2. Stress indicators of replicate releases should be monitored to ensure that groups of fish released four days apart do not vary widely in stress conditions.

3. Since smoltification may have been suppressed at Lyons Ferry SFH, as evidence by low gill Na^+K^+-ATPase levels, post-release increases in plasma thyroxine, and coloration; either the holding conditions should be changed or marking should be completed 1-2 days before release.

4. If the progressive increases in gill Na^+K^+-ATPase observed at McNary Dam are accepted as evidence of smoltification being a time related development after release, use of a control group transported directly from a hatchery and released a short distance above the recovery dam represents an insurmountable problem. Methods are not immediately available to overcome this biological problem, and therefore alternate approaches to survival estimates should be considered.

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Appendix la. Summary of mean (X) plasma cortisol ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Cortisol levels are basal values from fish at rest and one hour after a handling-stress challenge.

Lyons Ferry SFH	PRERELEASE				RELEASE			
Replicate Group	X	SD	CV	N	x	SD	CV	N
Rep 1 Control 1								
Basal	6.3	2.6	42	20	79.2	19.4	24	20
Handling	90.5	25.7	28	20	125.3	39.8	32	20
Test 1								
Basal	14.4	11.4	79	20	115.1	22.1	19	19
Handling	96.2	31.0	32	20	113.8	39.7	35	18
Rep 2 Control 2								
Basal	20.0	10.3	51	19	68.5	29.3	43	18
Handling	77.2	34.5	45	20	15.8	32.9	24	20
Test 2								
Basal	29.8	23.1	77	16	94.1	30.8	33	19
Handling	140.9	31.5	22	20	114.7	34.4	30	18
Rep 3 Control 3								
Basal	31.0	18.8	61	19	68.6	28.7	42	20
Handling	144.6	45.4	31	16	162.3	37.8	23	20
Test 3								
Basal	14.8	6.1	41	20	119.8	31.2	26	20
Handling	121.3	47.0	39	18	153.0	23.3	15	20

Appendix lb. Summary of two-way ANOVA on baseline cortisol data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified hy group (control and test) and by time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
Replicate = 1				
Group	1	8463.6	34.2	0.0001*
Time	1	147165.3	594.3	0.0001*
Group*Time	1	4752.3	19.2	0.0001*
Error	75	247.6		
Replicate = 2				
Group	1	7980.6	13.1	0.0006*
Time	1	58805.2	96.3	0.0001*
Group*Time	1	0.0	0.0	1 .0000
Error	68	610.9		
Replicate = 3				
Group	1	5731.1	10.4	0.0018*
Time	1	100923.8	183.9	0.0001*
Group*Time	1	23022.1	41.9	0.0001*
Error	75	548.9		

Appendix lc. Summary of two-way ANOVA on cortisol data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified by group (control and test) and challenqe (baseline and handling). Asterisk (*) denotes P < 0.05.

source	DF	Mean Square	F value	Pr>F
Replicate = 1, Time = prerelease				
Group	1	944.6	2.2	0.1468
Challenqe	1	137854.7	313.7	0.0001*
Group*Challenqe	1	29.2	0.1	0.7974
Error	76	439.5		
Replicate = 1, Time = release				
Group	1	2870.9	2.9	0.0940
Challenge	1	10390.8	10.4	0.0019*
Group*Challenqe	1	10944.4	11.0	0.0014*
Error	73	997.0		
Replicate = 2, Time = prerelease				
Group	1	33257.1	46.0	0.0001*
Challenge	1	133529.5	184.5	0.0001*
Group*Challenge	1	8117.8	11.2	0.0013*
Error	71	723.7		
Replicate = 2, Time = release				
Group	1	67.8	0.1	0.7972
Challenge	1	40056.6	39.3	0.0001*
Group*Challenqe	1	11926.6	11.7	0.0010*
Error	71	1019.5		
Replicate = 3, Time = prerelease				
Group	1	5693.6	5.2	0.0257*
Challenge	1	218101.0	199.2	0.0001*
Group*Challenge	1	1462.7	1.3	0.2517
Error	69	1094.9		
Replicate = 3, Time = release				
Group	1	8769.7	9.3	0.0031*
Challenge	1	80657.7	85.6	0.0001*
Group'Challenqe	1	18307.3	19.4	0.0001*
Error	76	941.9		

Appendix 2a. Summary of mean (X) plasma glucose ($mg \cdot dL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Glucose levels are basal values from fish at rest and one hour after a handling-stress challenge.

Lyons Ferry SF"		PRERELEASE				RELEASE			
Replicate	Group	X	SD	CV	N	X	SD	CV	N
Rep 1	Control 1								
	Basal	122.6	11.4	9	20	140.6	22.7	16	20
	Handling	148.9	28.1	19	20	126.6	47.5	37	19
	Test 1								
	Basal	115.1	16.1	14	20	151.4	16.9	11	19
	Handling	162.2	40.1	25	20	125.0	47.0	38	20
Rep 2	Control 2								
	Basal	138.2	17.0	12	19	130.0	24.5	19	19
	Handling	124.2	41.2	33	20	145.4	32.0	22	18
	Test 2								
	Basal	127.9	24.2	19	19	130.9	21.2	16	19
	Handling	163.1	25.5	16	20	135.3	47.8	35	20
Rep 3	Control 3								
	Basal	111.7	14.4	13	19	132.9	27.1	20	20
	Handling	158.5	29.5	19	16	120.0	26.4	22	20
	Test 3								
	Basal	139.7	23.3	17	20	145.0	18.6	13	20
	Handling	158.9	26.4	17	20	132.3	13.2	10	20

Appendix 2b. Summary of two-way ANOVA on baseline glucose data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified by group (control and test) and by time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
Replicate = 1				
Group	1	28.0	0.1	0.7595
Time	1	14405.4	48.5	0.0001*
Group*Time	1	1686.4	5.7	0.0197*
Error	75	297.1		
Replicate = 2				
Group	1	415.1	0.9	0.3557
Time	1	129.1	0.3	0.6058
Group*Time	1	607.0	1.3	0.2648
Error	72	480.5		
Replicate = 3				
Group	1	7719.1	16.8	0.0001*
Time	1	3314.2	7.2	0.0090*
Group*Time	1	1383.6	3.0	0.0871
Error	75	460.2		

Appendix 2c. Summary of two-way ANOVA on glucose data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified by group (control and test) and by challenge (baseline and handling). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr>F
Replicate = 1, Time = prerelease				
Group	1	171.4	0.2	0.6211
Challenge	1	26910.6	38.7	0.0001*
Group*Challenge	1	2188.9	3.2	0.0801
Error	76	695.1		
Replicate = 1, Time = release				
Group	1	327.3	0.2	0.6195
Challenge	1	7850.3	6.0	0.0170*
Group*Challenge	1	845.2	0.6	0.4255
Error	74	1316.3		
Replicate = 2, Time = prerelease				
Group	1	4349.3	5.3	0.0236*
Challenge	1	2176.3	2.7	0.1063
Group*Challenge	1	11821.1	14.5	0.0003*
Error	74	814.2		
Replicate = 2, Time = release				
Group	1	350.3	0.3	0.5755
Challenge	1	1762.7	1.6	0.2111
Group*Challenge	1	624.7	0.6	0.4550
Error	72	1107.0		
Replicate = 3, Time = prerelease				
Group	1	4905.9	8.4	0.0050*
Challenge	1	20051.8	34.4	0.0001*
Group*Challenge	1	2739.0	4.7	0.0336*
Error	71	583.4		
Replicate = 3, Time = release				
Group	1	2981.7	6.1	0.0156*
Challenge	1	3305.0	6.8	0.0110*
Group*Challenge	1	0.3	0.0	0.9790
Error	76	486.8		

Appendix 3a. Summary of mean (X1 gill N^+K^+-ATPase activity (umoles P_i • mg $protein^{-1} \cdot h^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Lyons Ferry SFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th, and 75th percentile (%) of miqration past the dam.

		Sample Site								
		Lyons Ferry SFH					McNary Dam			
Replicate	Group	X	SD	C"	N	%	X	SD	CV	N
Rep 1	Control 1	9.5	4.3	45	16	25	15.3	6.1	40	20
						50	20.3	4.1	20	20
						75	26.7	5.9	22	20
	Test 1	11.7	5.6	48	18	25	22.8	3.7	16	16
						50	28.9	5.1	18	20
						75	35.2	6.5	18	20
Rep 2	Control 2	10.4	4.0	38	14	25	12.2	2.3	19	20
						50	22.9	7.6	33	20
						75	26.4	6.4	24	8
	Test 2	9.6	3.3	34	19	25	20.0	5.4	27	20
						50	28.3	5.6	20	20
						75	33.9	10.9	32	20
Rep 3	Control 3	9.7	4.3	44	18	25	9.1	2.6	29	19
						50	14.4	3.8	26	20
						75	21.4	4.5	21	20
	Test 3	9.4	3.4	36	19	25	21.6	3.2	15	20
						50	26.1	5.1	19	20
						75	33.6	11.5	34	20

Appendix 3b. Summary of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1, 2, and 3). Asterisk (*) denotes P < 0.05.

source	DF	Mean square	F value	Pr>F
Group	1	4.2	0.2	0.6341
Replicate	2	9.3	0.5	0.6066
Group*Replicate	2	24.9	1.4	0.2642
Error	99	18.5		

Appendix 3c. Summary Of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile steelhead from Lyons Ferry SFH collected at McNary Dam during spring 1987. Data were classified by group (control and test) and by percentile of migration (25,50, and 75th). Asterisk (*) denotes $P < 0.05$.

SOURCE	DF	Mean Square	F value	Pr>F
Replicate = 1				
Group	1	2153.1	70.7	0.0001*
Percentile	2	1424.4	46.8	0.0001*
Group*Percentile	2	0.0	0.0	1.0000
Error	110	30.5		
Replicate = 2				
Group	1	1843.4	36.3	0.0001*
Percentile	2	2105.9	41.5	0.0001*
Group*Percentile	2	0.0	0.0	.oooo
Error	102	50.8		
Replicate = 3				
Group	1	4306.8	113.5	0.0001*
Percentile	2	1461.1	38.5	0.0001*
Group*Percentile	2	35.9	.o	0.3913
Error	113	37.9		

Appendix 4a. Summary of mean (X) plasma thyroxine ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Lyons Ferry SFH. Samples were collected at the hatchery and from freeze branded fish recaptured at McNary Dam during spring, 1987.

Lyons Ferry SFH	Sample Site Hatchery				Sample Site McNary Dam			
Group	x	SD	cv	N	X	SD	cv	N
Replicate 1								
Control 1	3.5	1.5	44	19	7.0	4.9	70	30
Test 1	3.7	2.0	53	19	4.8	2.1	43	30
Replicate 2								
Control 2	4.1	1.9	47	19	8.0	5.8	73	28
Test 2	3.8	1.9	49	20	6.9	6.7	98	30
Replicate 3								
Control 3	4.1	2.6	63	19	11.9	7.6	64	30
Test 3	2.6	1.2	45	18	7.4	4.8	64	30

Appendix 4b. Summary of two-way ANOVA on thyroxine data from juvenile steelhead from Lyons Ferry SFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	PT>F
Sample Site = Hatchery				
Group	1	6.4	1.8	0.1811
Replicate	2	3.5	1.0	0.3797
Group*Replicate	2	7.4	2.1	0.1286
Error	108	3.5		
Sample Site = McNary Dam				
Group	1	303.4	9.7	0.0021*
Replicate	2	211.7	6.8	0.0015*
Group*Replicate	2	45.3	1.4	0.2370
Error	172	31.2		

Appendix 5a. Summary of mean (X) fork length (mm), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Lyons Ferry SFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th, and 75th percentile (%) of migration past the dam.

		Sample Site								
		Lyons Ferry SFH					McNary Dam			
Replicate	Group	x	SD	CV	N	%	x	SD	cv	N
Rep 1	Control 1	199	14.5	7	300	25	205	14.7	7	21
						50	204	10.2	5	22
						75	210	13.5	6	21
	Test 1	200	14.3	7	300	25	209	12.6	6	19
						50	213	12.5	6	21
						75	210	10.7	5	21
Rep 2	Control 2	197	16.1	8	300	25	202	10.8	5	21
						50	207	10.0	5	21
						75	206	7.4	4	8
	Test 2	195	14.3	7	300	25	204	10.7	5	22
						50	209	13.5	6	20
						75	205	12.6	6	20
Rep 3	Control 3	199	13.9	7	300	25	206	7.4	4	20
						50	205	9.1	4	25
						75	209	8.9	4	21
	Test 3	199	13.3	7	300	25	205	11.3	6	20
						50	209	10.9	5	22
						75	211	15.5	7	20

Appendix 5h. Summary of two-way ANOVA on fork length of juvenile steelhead from Lyons Ferry SFH during spring, 1987. Data were classified by group (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr > F
Group	1	285.6	1.6	0.2065
Replicate	2	1932.5	10.8	0.0001*
Group*Replicate	2	67.7	0.4	0.6847
Error	594	178.6		

Appendix 6a. summary of mean (X) plasma cortisol ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Wells SFH during April and May, 1987. Cortisol levels are basal valves from fish at rest and one hour after a handling-stress challenge.

Wells SFH		PRERELEASE				RELEASE			
Replicate	Group	X	SD	CV	N	X	SD	CV	N
Rep 1	Control 1								
	Basal	76.6	59.5	78	18	95.6	53.5	56	17
	Handling	146.2	23.3	16	20	158.5	26.3	17	18
	Test 1								
	Basal	76.0	42.8	56	10	114.5	55.5	48	20
	Handling	148.3	35.1	24	18	143.6	27.8	19	20
Rep 2	Control 2								
	Basal	58.2	24.7	60	15	76.2	48.7	64	20
	Handling	136.4	36.4	27	19	127.8	31.6	25	20
	Test 2								
	Basal	73.8	28.2	38	20	105.4	29.8	28	20
	Handling	140.5	32.1	23	20	115.4	57.1	49	18
Rep 3	Control 3								
	Basal	45.2	30.1	67	19	65.8	30.3	46	20
	Handling	128.5	40.7	32	19	146.1	28.4	19	19
	Test 3								
	Basal	22.4	20.1	90	18	120.0	69.2	58	29
	Handling	145.3	31.8	22	20	138.8	38.6	28	20

Appendix 6h. Summary of two-way ANOVA on baseline cortisol data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified by (control and test) and hy time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
Replicate = 1				
Group	1	4308.0	1.4	0.2480
Time	1	13784.2	4.6	0.0351*
Group*Time	1	0.0	0.0	1 .0000
Error	61	2967.9		
Replicate = 2				
Group	1	8321.8	6.3	0.0144*
Time	1	10453.3	7.9	0.0064*
Group*Time	1	2278.8	1.7	0.1933
Error	71	1321.4		
Replicate = 3				
Group	1	5358.6	3.0	0.0852
Time	1	64091.3	36.4	0.0001*
Group*Time	1	28100.3	16.0	0.0002*
Error	72	1759.2		

Appendix 6c. Summary of two-way ANOVA on cortisol data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified by group (control and test) and by challenge (baseline and handling). Asterisk (*) denotes P < 0.05.

source	DF	Mean square	F Value	Pr > F
Replicate = 1, Time = prerelease				
Group	1	1378.4	0.8	0.3770
Challenge	1	08689.7	46.4	0.0001*
Group*Challenqe	1	0.0	0.0	1 .0000
Error	62	1740.6		
Replicate = 1, Time = release				
Group	1	21.7	0.0	0.9139
Challenge	1	37709.0	20.5	0.0001*
Group*Challenge	1	5340.8	2.9	0.0930
Error	71	1841.7		
Replicate = 2, Time = prerelease				
Group	1	511.9	0.5	0.4928
Challenge	1	93997.3	87.3	0.0001*
Group*Challenge	1	1750.2	1.6	0.2065
Error	70	1076.9		
Replicate = 2, Time = release				
Group	1	1278.0	0.7	0.4077
Challenge	1	18852.0	10.2	0.0020*
Group*Challenge	1	8690.7	4.7	0.0331*
Error	74	1843.0		
Replicate = 3, Time = prerelease				
Group	1	1.4	0.0	0.9701
Challenge	1	201389.7	200.8	0.0001*
Group*Challenge	1	7547.5	7.5	0.0077*
Error	72	1002.8		
Replicate = 3, Time = release				
Group	1	11904.2	6.0	0.0165*
Challenge	1	49050.5	24.8	0.0001*
Group*Challenge	1	17297.5	8.8	0.0042*
Error	74	1977.9		

Appendix 7a. Summary of mean (X) plasma glucose ($mg \cdot dL^{-1}$), standard deviation (SD) coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Wells SFH during April and May, 1987. Glucose levels are basal valves from fish at rest and one hour after a handling-stress challenqe.

Wells SFH		PRERELEASE				RELEASE			
Replicate	Group	X	SD	CV	N	X	SD	cv	N
Rep 1	Control 1								
	Basal	171.2	107.7	63	18	198.0	89.1	45	17
	Handling	200.0	100.7	50	20	189.0	78.5	42	18
	Test 1								
	Basal	213.5	178.8	84	8	190.4	42.0	38	20
	Handling	222.2	93.0	42	17	189.6	84.3	44	20
Rep 2	Control 2								
	Basal	109.2	31.7	29	15	165.2	52.8	32	20
	Handling	114.4	42.3	37	19	153.3	56.9	37	20
	Test 2								
	Basal	101.0	39.9	39	20	130.5	63.8	48	20
	Handling	103.9	13.9	13	20	123.6	29.2	24	18
Rep 3	Control 3								
	Basal	109.4	14.8	13	19	129.1	20.2	16	20
	Handling	122.1	24.2	20	19	133.6	32.5	24	19
	Test 3								
	Basal	92.3	16.2	18	17	135.3	34.5	25	19
	Handling	114.2	28.0	24	20	118.8	43.8	37	20

Appendix 7h. Summary of two-way ANOVA on baseline glucose data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified by qroup (control and test) and by time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr>F
Replicate = 1				
Group	1	2552.0	0.2	0.6312
Time	1	1429.3	0.4	0.7193
Group*Time	1	7878.4	0.7	0.4000
Error	59	10961.5		
Replicate = 2				
Group	1	12078.7	5.0	0.0288*
Time	1	35084.3	14.5	0.0003*
Group*Time	1	511.8	0.2	0.6473
Error	71	2424.1		
Replicate = 3				
Group	1	380.7	0.7	0.3977
Time	1	17739.4	33.7	0.0001*
Group*Time	1	2619.0	5.0	0.0288*
Error	71	525.8		

Appendix 7c. Summary of two-way ANOVA on glucose data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified by group (control and test) and by challenge (baseline and handling). Asterisk (*) denotes P < 0.05.

source	DF	Mean square	F value	Pr > F
Replicate = 1, Time = prerelease				
Group	1	16462.8	1.3	0.2604
Challenge	1	10307.1	0.8	0.3722
Group*Challenge	1	0.0	0.0	1.0000
Error	59	12748.4		
Replicate = 1, Time = release				
Group	1	201.5	0.0	0.8613
Challenge	1	401.0	0.1	0.8054
Group*Challenge	1	327.4	0.1	0.8238
Error	71	6557.7		
Replicate = 2, Time = prerelease				
Group	1	1171.8	1.5	0.2257
Challenge	1	378.2	0.3	0.5675
Group*Challenge	1	0.0	0.0	1.0000
Error	70	1145.8		
Replicate = 2, Time = release				
Group	1	19963.6	7.2	0.0088*
Challenge	1	1452.9	0.5	0.4699
Group*Challenge	1	417.0	0.2	0.6983
Error	74	2754.2		
Replicate = 3, **Time = prerelease**				
Group	1	2525.2	5.4	0.0236*
Challenge	1	5248.3	11.1	0.0014*
Group*Challenge	1	705.6	1.5	0.2255
Error	71	471.9		
Replicate = 3, Time = release				
Group	1	305.0	0.3	0.5635
Challenge	1	725.6	0.6	0.4282
Group*Challenge	1	2118.75	1.8	0.1776
Error	74	1143.5		

Appendix 8a. Summary of mean (X) qill Na^+K^+-ATPase activity (umoles P$_i$ • mq protein^{-1}•h^{-1}), standard deviation (SD), coefficient of variatton (CV in percent), and sample size (N) of juvenile steelhead from Wells SFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th, and 75th percentile (%) of miqration past the dam.

		Sample Site								
		Wells SFH					McNary Dam			
Replicate	Group	X	SD	CV	N	%	X	SD	CV	N
Rep 1	Control 1	14.7	5.6	38	20	25	24.8	5.7	23	19
						50	29.5	8.1	27	20
						75	30.5	9.4	31	20
	Test 1	15.0	4.5	30	19	25	26.0	5.6	21	20
						50	30.6	7.6	25	20
						75	33.2	8.7	26	20
Rep 2	Control 2	18.2	5.9	32	20	25	19.1	3.6	19	20
						50	23.1	5.5	24	20
						75	31.7	10.9	34	20
	Test 2	16.5	4.5	27	20	25	31.7	5.3	17	20
						50	30.7	6.7	22	20
						75	36.9	9.5	26	20
Rep 3	Control 3	20.1	6.0	30	20	25	17.9	5.7	32	20
						50	22.9	5.8	25	20
						75	25.8	6.9	27	18
	Test 3	19.6	7.4	38	19	25	26.3	7.2	27	20
						50	36.3	8.4	23	20
						75	38.2	10.9	28	20

Appendix 8b. Summary of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr>F
Group	1	11.6	0.3	0.5632
Replicate	2	243.4	7.0	0.0013*
Group*Replicate	2	9.3	0.3	0.7639
Error	112	34.5		

Appendix 8c. Summary of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile steelhead from Wells SFH collected at McNary Dam during spring, 1987. Data were classified by group (control and test) and by percentile of migration (25,50, and 75th). Asterisk (*) denotes $P < 0.05$.

Source	DF	Mean Square	F value	Pr>F
Replicate = 1				
Group	1	74.7	1.2	0.2730
Percentile	2	428.6	7.0	0.0014*
Group*Percentile	2	10.7	0.2	0.8410
Error	113	61.6		
Replicate = 2				
Group	1	2132.3	37.6	0.0001*
Percentile	2	905.4	16.0	0.0001*
Group*Percentile	2	144.6	2.6	0.0828
Error	114	56.8		
Replicate = 3				
Group	1	3945.8	63.2	0.0001*
Percentile	2	1103.7	17.7	0.0001*
Group*Percentile	2	19.8	0.3	0.7285
Error	112	62.4		

Appendix 9a. Summary of mean (X) plasma thyroxine ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Wells SFH. Samples were collected at the hatchery and from freeze branded fish recaptured at McNary Dam during spring, 1987.

Wells SFH	Sample Site Hatchery				Sample Site McNary Dam			
Group	X	SD	CV	N	X	SD	CV	N
Replicate 1								
Control 1	6.2	4.5	73	15	4.1	1.9	47	29
Test 1	4.2	4.1	97	12	3.3	1.5	45	30
Replicate 2								
Control 2	3.5	2.1	62	19	4.0	2.4	59	30
Test 2	7.1	4.4	62	20	4.8	2.4	50	30
Replicate 3								
Control 3	3.5	1.7	49	20	4.2	2.2	51	30
Test 3	6.9	6.2	90	19	5.2	3.5	68	30

Appendix 9b. Summary of two-way ANOVA on thyroxine data from juvenile steelhead from Wells SFH during April and May, 1987. Data were classified hy group (control and test) and hy replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
---------- Sample Site = Hatchery ----------				
Group	1	112.8	6.8	0.0108*
Replicate	2	0.4	0.0	0.9756
Group*Replicate	2	74.4	4.5	0.0140*
Error	99	16.7		
---------- Sample Site = McNary Dam ----------				
Group	1	4.2	0.7	0.3907
Replicate	2	16.0	2.8	0.0620
Group*Replicate	2	14.6	2.6	0.0788
Error	173	5.7		

Appendix 10a. Summary of mean (X) fork lenqth (mm), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile steelhead from Wells SFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th. and 75th percentile (%) of migration past the dam.

		sample Site								
		Wells SFH					McNary Dam			
Replicate	Group	X	SD	CV	N	%	X	SD	CV	N
Rep 1	Control 1	195	16.2	8	300	25	201	13.9	7	20
						50	209	16.4	8	22
						75	208	18.0	9	22
	Test 1	190	21.2	11	282	25	202	18.7	9	22
						50	202	14.2	7	21
						75	205	19.7	10	22
Rep 2	Control 2	197	14.2	7	300	25	200	14.0	7	20
						50	207	14.4	7	20
						75	195	13.0	7	21
	Test 2	192	20.9	11	327	25	202	12.2	6	20
						50	202	15.8	8	20
						75	207	13.4	6	21
Rep 3	Control 3	196	15.6	8	305	25	196	11.6	6	22
						50	201	9.0	4	22
						75	199	11.3	6	18
	Test 3	193	19.4	10	305	25	202	15.7	8	21
						50	205	17.1	8	20
						75	210	13.4	6	20

Appendix 10b. Summary of two-way ANOVA on fork length of juvenile steelhead from Wells SFH durinq spring, 1987. Data were classified by (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr > F
Group	1	913.8	3.8	0.0533
Replicate	2	496.0	2.0	0.1316
Group*Replicate	2	189.9	0.8	0.4593
Error	592	243.8		

Appendix lla. Summary of mean (X) plasma cortisol ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile spring chinook salmon from Winthrop NFH during April and May, 1987. Cortisol levels are basal values from fish at rest and one hour after a handling-stress challenge.

Winthrop NFH		PRERELEASE				RELEASE			
Replicate	Group	x	SD	cv	N	x	SD	CV	N
Rep 1	Control 1								
	Basal	6.6	6.0	91	19	79.9	48.6	61	16
	Handling								
	Test 1								
	Basal	15.5	9.9	64	19	14.8	10.6	72	22
	Handling								
Rep 2	Control 2								
	Basal	22.1	14.8	67	18	89.4	46.0	51	18
	Handling	140.7	20.3	14	20	145.4	26.9	18	17
	Test 2								
	Basal	9.5	7.4	78	20	13.9	12.3	89	18
	Handling	107.1	20.0	19	17	111.2	34.4	31	19
Rep 3	Control 3								
	Basal	39.6	47.0	119	13	72.9	25.5	35	11
	Handling	142.4	46.5	33	18	135.5	38.7	29	15
	Test 3								
	Basal	74.0	40.5	55	15	48.5	24.6	51	IS
	Handling	101.9	47.7	48	19	120.8	30.0	25	14

Appendix llh. Summary of two-way ANOVA on baseline cortisol data from juvenile spring chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by (control and test) and by time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr>F
Replicate = 1				
Group	1	11805.7	21.2	0.0001*
Time	1	18498.5	33.2	0.0001*
Group*Time	1	28196.9	50.6	0.0001*
Error	72	557.2		
Replicate = 2				
Group	1	36078.2	58.3	0.0001*
Time	1	24157.8	39.0	0.0001*
Group*Time	1	16821.8	27.2	0.0001*
Error	70	619.1		
Replicate = 3				
Group	1	542.3	0.4	0.5196
Time	1	8.0	0.0	0.9375
Group*Time	1	11443.2	8.9	0.0045*
Error	50	1289.5		

Appendix 11c. Summary of two-way ANOVA on cortisol data from juvenile spring chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and by challenge (baseline and handling). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
---------- Replicate = 2 Time = prerelease ----------				
Group	1	17084.5	63.8	0.0001*
Challenge	1	225853.6	843.4	0.0001*
Group*Challenge	1	0.0	0.0	1.0000
Error	71	267.8		
---------- Replicate = 2, Time = release ----------				
Group	1	50040.1	47.7	0.0001*
Challenge	1	103194.2	98.3	0.0001*
Group*Challenge	1	11828.4	11.2	0.0013*
Error	68	1049.4		
---------- Replicate = 3, Time = prerelease ----------				
Group	1	1537.4	0.7	0.3937
Challenge	1	64489.0	31.0	0.0001*
Group*Challenge	1	21863.0	10.5	0.0019*
Error	61	2083.5		
---------- Replicate = 3, Time = release ----------				
Group	1	9003.8	9.6	0.0031*
Challenge	1	66411.3	71.0	0.0001*
Group*Challenge	1	0.0	0.0	1.0000
Error	51	935.8		

Appendix 12a. Summary of mean (X) plasma glucose ($mg \cdot dL^{-1}$), standard deviation (SD) coefficient of variation (CV in percent), and sample size (N) of juvenile spring chinook salmon from Winthrop NFH duriny April, 1987. Glucose levels are basal valves from fish at rest and one hour after a handling-stress challenge.

Winthrop NFH		PRERELEASE				RELEASE			
Replicate	Group	X	SD	cv	N	X	SD	cv	N
Rep 1	Control 1								
	Basal	76.4	26.2	34	19	73.3	29.0	40	12
	Handling								
	Test 1								
	Basal	87.2	14.6	17	20	99.0	16.4	17	23
	Handling								
Rep 2	Control 2								
	Basal	84.1	32.0	38	18	03.9	25.9	31	17
	Handling	76.8	11.6	15	20	88.4	34.2	39	20
	Test 2								
	Basal	89.7	28.7	32	20	87.3	13.6	15	18
	Handling	93.0	19.5	21	17	101.6	20.9	21	20
Rep 3	Control 3								
	Basal	89.1	31.4	35	13	171.3	157.7	92	10
	Handling	159.4	105.2	66	18	83.3	22.6	27	15
	Test 3								
	Basal	110.0	53.0	48	16	87.1	10.0	11	15
	Handling	124.9	38.6	31	19	118.6	24.5	21	14

Appendix 12b. Summary of two-way ANOVA on baseline glucose data from juvenile chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and time (prerelease and release). Asterisk (*) denotes P < 0.05.

Source	DF	Mean square	F value	Pr>F
		Replicate = 1		
Group	1	6022.8	13.4	0.0005*
Time	1	1253.8	2.8	0.0999
Group*Time	1	307.9	0.7	0.4115
Error	70	451.0		
		Replicate = 2		
Group	1	380.1	0.6	0.4573
Time	1	36.1	0.1	0.8184
Group*Time	1	18.8	0.0	0.8686
Error	69	680.3		
		Replicate = 3		
Group	1	8884.8	1.6	0.2129
Time	1	5435.8	1.0	0.3284
Group*Time	1	36767.9	6.6	0.0133*
Error	50	5581.2		

Appendix 12c. Summary of two-way ANOVA on glucose data from juvenile spring chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and by challenge (baseline and handling). Asterisk (*) denotes $P < 0.05$.

Source	DF	Mean square	F value	Pr > F
Replicate = 2, Time = prerelease				
Group	1	2239.7	3.8	0.0549
Challenge	1	152.1	0.3	0.6125
Group*Challenge	1	451.2	0.8	0.3838
Error	71	587.7		
Replicate = 2, Time = release				
Group	1	1347.1	2.1	0.1465
Challenge	1	1619.1	2.6	0.1119
Group*Challenge	1	484.3	0.8	0.3816
Error	71	624.9		
Replicate = 3, Time = prerelease				
Group	1	2311.9	0.5	0.4682
Challenge	1	21443.2	6.3	0.0145*
Group*Challenge	1	11821.3	2.7	0.1039
Error	62	4338.9		
Replicate = 3, Time = release				
Group	1	3535.6	0.7	0.3949
Challenge	1	5591.5	1.2	0.2857
Group*Challenge	1	47987.8	10.0	0.0027*
Error	50			

Appendix 13a. Summary of mean (X) gill Na^+K^+-ATPase activity (umoles P_i · mg protein^{-1}·h^{-1}), standard deviation (SD), coefficient of variation (CV in percent), and sample size (NJ of juvenile spring chinook salmon from Winthrop NFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th and 75th percentile (%) of migration past the dam.

		Sample Site								
		Winthrop NFH					McNary Dam			
Replicate	Group	X	SD	CV	N	%	X	SD	CV	N
Rep 1	Control 1	15.6	3.9	25	20	25	24.1	6.0	25	18
						50	26.2	4.7	18	18
						75	32.3	5.7	18	19
	Test 1	18.2	4.6	25	19	25	31.4	8.7	28	20
						50	30.5	8.6	28	20
						75	30.6	7.0	23	19
Rep 2	Control 2	15.9	3.8	24	17	25	23.0	6.0	26	19
						50	25.3	5.5	22	15
						75	30.4	7.3	24	20
	Test 2	16.9	4.1	24	20	25	28.3	6.9	24	20
						50	31.3	5.8	18	20
						75	28.1	7.5	27	20
Rep 3	Control 3	16.3	4.6	28	20	25	16.8	4.4	26	20
						50	22.1	4.3	19	18
						75	26.6	6.1	23	20
	Test 3	18.2	4.6	25	19	25	28.7	7.3	25	19
						50	32.4	6.5	20	20
						75	25.6	7.0	27	20

Appendix 13b. Summary of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile spring chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1,2 and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr>F
Group	1	18.3	1.1	0.3028
Replicate	2	11.4	0.7	0.5136
Group*Replicate	2	33.1	1.9	0.1486
Error	112	17.1		

Appendix 13c. Summary of two-way ANOVA on gill Na^+K^+-ATPase data from juvenile spring chinook salmon from Winthrop NFH collected at McNary Dam during spring, 1987. Data were classified by group (control and test) and by percentile of migration (25.50, and 75th). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr > F
Replicate = 1				
Group	1	297.3	5.8	0.0176*
Percentile	2	131.2	2.6	0.0816
Group*Percentile	2	209.3	4.1	0.0194*
Error	109	51.2		
Replicate = 2				
Group	1	239.2	5.2	0.0242*
Percentile	2	147.0	3.2	0.0441*
Group*Percentile	2	202.1	4.4	0.0143*
Error	109	45.8		
Replicate = 3				
Group	1	1481.0	38.1	0.0001*
Percentile	2	252.1	6.5	0.0022*
Group*Percentile	2	462.7	11.9	0.0001*
Error	111	39.9		

Appendix 14a. Summary of mean (X) plasma thyroxine ($ng \cdot mL^{-1}$), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile spring chinook salmon from Winthrop NFH. Samples were collected at the hatchery and from freeze branded fish recaptured at McNary Dam during spring, 1987.

Winthrop NFH	Sample Site Hatchery				Sample Site McNary Dam			
Group	X	SD	cv	N	X	SD	cv	N
Replicate 1								
Control 1	4.0	1.3	32	17	6.8	3.0	44	28
Test 1	2.8	1.0	38	19	4.7	2.4	51	29
Replicate 2								
Control 2	3.9	1.6	40	16	4.8	2.7	56	30
Test 2	4.8	1.6	33	20	11.2	4.3	38	30
Replicate 3								
Control 3	6.7	3.5	52	17	6.8	2.4	36	30
Test 3	6.0	2.8	46	18	6.7	3.3	49	30

Appendix 14b. Summary of one-way ANOVA on thyroxine data from juvenile chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr > F
Sample Site = Hatchery				
Group	1	4.1	0.9	0.3352
Replicate	2	80.8	18.1	0.0001*
Group*Replicate	2	9.4	2.1	0.1272
Error	101	4.5		
Sample Site = McNary Dam				
Group	1	96.9	10.2	0.0017*
Replicate	2	73.7	7.7	0.0006*
Group*Replicate	2	297.6	36.1	0.0001*
Error	171	9.5		

Appendix 15a. Summary of mean (X) fork length (mm), standard deviation (SD), coefficient of variation (CV in percent), and sample size (N) of juvenile spring chinook salmon from Winthrop NFH and at McNary Dam during spring, 1987. Samples of freeze branded fish were recaptured at approximately 25th, 50th, and 75th percentile (%) of migration past the dam.

			Sample Site								
			Winthrop NFH					McNary Dam			
Replicate	Group		X	SD	CV	N	%	X	SD	CV	N
Rep 1	Control	1	124	9.0	7	296	25	135	11.9	16	20
							50	134	11.2	8	20
							75	134	5.5	4	19
	Test	1	132	9.4	7	303	25	141	12.2	9	23
							50	140	8.8	6	21
							75	148	7.7	5	20
Rep 2	Control	2	124	11.1	9	291	25	126	9.7	8	21
							50	128	11.2	9	17
							75	133	9.6	7	20
	Test	2	133	9.7	7	300	25	140	8.6	6	20
							50	144	8.2	6	22
							75	158	6.6	4	20
Rep 3	Control	3	124	11.6	9	300	25	124	17.9	14	23
							50	127	7.5	6	20
							75	128	12.1	9	25
	Test	3	134	10.9	8	299	25	148	11.3	8	19
							50	148	10.3	7	21
							75	157	7.5	5	20

Appendix 15b. Summary of two-way ANOVA on fork length of juvenile fall chinook salmon from Winthrop NFH during April and May, 1987. Data were classified by group (control and test) and by replicate (1,2, and 3). Asterisk (*) denotes P < 0.05.

Source	DF	Mean Square	F value	Pr > F
Group	1	11574.3	98.8	0.0001*
Replicate	2	75.2	0.6	0.5266
Group*Replicate	2	90.1	0.8	0.4619
Error	598	117.2		